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THE EFFECT OF CASTRATION ON THE WEIGHT OF THE PITUITARY BODY AND OTHER GLANDS OF INTERNAL SECRETION IN THE RABBIT

A. E. LIVINGSTON

From the Physiological Laboratory, Cornell University, Ithaca, N. Y.

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INTRODUCTION

The object of the present investigation was to determine the effect produced on the weight of the hypophysis by removing the testes and ovaries from male and female rabbits. It has been reported that the anterior lobe of the pituitary is affected in weight and histological appearance by castration. The same part of the pituitary is generally considered to be in some way associated with body growth. Castration in certain cases has been shown without a doubt to affect growth of animals. With these facts in mind the idea was conceived of utilizing the same animals for a determination of the effect of castration and spaying on the body growth and also the effect on weight or histological appearance of some other glands of internal secretion.

It is probable that the operation which consists of removing the testes from male animals has been practiced longer than any other. Many of the effects have been noticed from the very first and some are still disputed questions. The results most manifest are upon the secondary sex organs and characters; the whole body, however, undergoes changes in metabolism and development. Most of these changes are at best only vaguely understood and of others practically nothing is known. In mammals the most marked change is in the prostate and seminal vesicles. If their development is not complete it ceases, and if complete, atrophy results (Griffiths (9)). The effects which follow

removal of the ovaries from females are just as distinct, and are probably less understood than those peculiar to males. Some of the results are common to both sexes.

It was not until Brown-Sequard (2) in 1889 published his results that serious investigation began in this field. His findings, although probably not of so much value in themselves, suggested what is to be the basis of explanation for most if not all the effects following the removal of sex organs from both males and females, namely an internal secretion. It seems quite reasonable to suppose that all organs which produce an internal secretion are more or less intimately related to each other and for this reason it is difficult to determine the direct effect upon any one of these by removal or alteration in the normal action of any other.

In certain respects the pituitary gland is considered by some writers to be more closely related to the testes and ovaries than to any other of the glands of internal secretion. Experimentally the relationship between any two of these organs has been studied usually by one or more of four methods, (1) by removing one of the two glands in question and observing the effect produced upon the other in the form of an increase or decrease in weight, histological changes, external appearance and so on; (2) by removing the other gland and noting the reverse effects; (3) by administering to the experimental animal in some form the substance of the gland which in the other cases was taken away; and (4) by reversing this procedure by feeding the substance of the gland upon which the effect was watched in the preceding cases. Another method which can scarcely be placed in any one of these four classes is to remove an organ from its natural position and graft it into some other part of the body. This method has been used to support the theory of internal secretions in the case of the testes of fowls (Foges (8)) and many other forms. In addition to being studied experimentally they have been approached from the field of pathology, from clinical records, and from the results of operations upon the human subject.

Advantage has been taken of most of these methods for adding to our knowledge concerning the relationship between the pituitary and the generative glands. Several investigators have removed the testes or ovaries from various animals under different conditions and have used different methods for determining the effect upon the weight of the hypophysis or other organs. The animals used have included rabbits, guinea pigs, cocks, bulls, buffaloes, sows, ewes, rats, horses and in addition observations on eunuchs. The conclusions thus far reached have

been contradictory so that at the present time the question is obviously an open one.

With the object of the present experiment in mind, precaution has been taken to avoid all errors which might possibly affect the results. Two series of animals each composed of both sexes have been used.

PREVIOUS WORK

Comparatively few investigators have studied this problem directly. Much however has been written on subjects closely associated with this question and for a complete survey of the literature the reader may consult Biedl (1), D. Noel Paton (20), and Swale Vincent (24). Only those whose results are most closely related to this subject will be mentioned here.

The first and probably the most widely quoted worker in this field is Fichera (7) who observed the weight and histological appearance of the pituitary bodies of 50 cocks and 50 capons, 2 normal and 3 spayed rabbits, 2 normal and 3 spayed guinea pigs, 5 normal and 5 castrated buffaloes and 5 normal and 5 castrated bulls. The figures which he gives include for each group the average, minimal, and maximal weight of the hypophysis in centigrams, for both control and experimental animals. Fichera concludes from these experiments that the pituitary enlarges to about twice the normal size after castration, and also reports that he was able to rapidly reduce this enlargement by the injection of testicular extract. He believes that there is some sort of compensatory relationship between the genitals and the hypophysis. It seems entirely possible however that his figures do not show what might be found if some of his sources of error were eliminated. With the exception of the cocks and capons the number of animals is too small to be taken as conclusive proof when such great individual variations are obtained normally. The body weight of the animals is not given in any case; thus his results are not given in terms of body weight, which obviously might make an appreciable difference. In the case of rabbits and guinea pigs, where females were used, no mention is made as to whether or not the animals had ever been pregnant, and this condition is claimed by some investigators to cause an enlargement of the pituitary gland which probably returns to normal during lactation but may persist for a longer time. The animals are not said to be of the same litter which would also be apt to cause a variation. In case of some animal species, especially those in which the gastro-intestinal con-

tents may vary between comparatively wide limits in proportion to body weight, it seems necessary to also avoid this possible source of error.

In regard to the condition of the pituitary during pregnancy as just mentioned, Comte (4) seems to have been the first to report an enlargement, and later many others have come to the same conclusion. Erdheim and Stumme (6) have produced indisputable evidence of hypophyseal hypertrophy during pregnancy and of changes which take place in the structure of the organ, similar to those which Fichera, as just mentioned, has described as resulting from castration.

As to the necessity of taking into account the weight of the gastrointestinal contents in the case of the rabbit, as here considered, reference should be made to some results of Joseph (15) in which the content weight is on the average about 10 per cent of body weight and varies between comparatively wide limits. The same is shown to be true in case of the rabbits used in the present experiment as already published (17).

Cimorini (3) confirms the work of Fichera by using young dogs and states that the changes which take place are similar to those occurring after removal of the thyroids. Tandler and Gross (23) show that there was an anatomical enlargement of the sella turcica by examination of the skulls of skeletons and by means of X-ray photographs of living castrated animals.

The work of Marrassini and Luciani (19) owing to its scope and character undoubtedly deserves careful consideration. Their observations were made on sheep, cattle, dogs, rabbits, guinea pigs and domestic fowls. The age, time after castration, weight, and in most cases the relation of the animals were known, i.e., whether they belonged to the same or different litters. They give not only the gross weight of the animal, the individual weight of each hypophysis and their relative weights, but also the weight of each brain, kidney, heart, suprarenal gland, liver, spleen, pancreas, thyroid, and the normal generative glands. They conclude that in sheep and cattle the weight of the hypophysis, which normally presents wide individual variations seems not to have undergone modifications capable of constituting a particular characteristic of castrated animals. Although no weights with respect to dogs are given in their tables the statement is made that the weight of the hypophysis in proportion to body weight does not show any constant difference which merits special attention. Among rabbits and guinea pigs either male or female they do not observe after

castration any constant modification deserving mention. The same is said of those males in which a bilateral ligature of the ductus deferens was made, and a ligature of the ductus deferens on one side and castration on the other side. They further say that the difference which one sometimes observes in the weight of the hypophysis as well as in all other organs must, in all probability, depend on special individual conditions, not attributable directly to the suppression or the modification of the function of the sexual glands.

D. Noel Paton (20) in discussing this question, considers it to be proven that hypertrophy of the pituitary is caused by castration as well as by thyroidectomy and is inclined to consider this as an indication that there is a reciprocal relationship between these organs. On the gonads the pituitary is generally believed to have an augmentary action; both have an important influence on growth and development, and when the gonads are removed the pituitary is regarded by some as undergoing what is thought to be a compensatory hypertrophy. It is stated that without the pituitary, complete development of the gonads appears impossible.

Cushing (5) reports that in the case of the majority of adult dogs from which the posterior lobe of the pituitary had been removed the females did not come in heat even when observed for nine months, and the males ultimately showed definite testicular atrophy. The puppies of both sexes remain sexually infantile.

Kuhn (16) examined the pituitaries of castrated horses, mares and stallions. Owing to the difficulty of obtaining stallions for examination he was able to report the pituitary weight of only two. His conclusions are therefore mainly based on the comparison of castrated males and normal females. The sex difference in the horse is not regarded as an appreciable factor. The females used in the investigation had never been pregnant. He concludes that the pituitaries of horses do not respond by an increase in size or volume after removal of the testes. He points to the wide individual variation which he noticed among those of the normal groups and attributes the slight difference between the operated and control animals, which is in favor of the normals, to this cause rather than to any effect of castration.

Hatai (10) reports a distinct sex difference when comparing the rate of increase in weight of the hypophysis during growth in male and female albino rats. This sex difference was noticeable in rats which weighed 50 grams, and became more noticeable as the animals increased in weight. The hypophysis in the adult female was more than twice as

heavy as in the male in proportion to body weight. In a later publication (11) he states that in the case of the Norway rat this sex difference is very slight and he questions whether or not it is present at all in guinea pigs and rabbits as shown by the results of other investigators. No explanation is given for the sex difference in the case of the albino rat but it is made clear by his results that the hypophysis reacts differently according to sex after castration and spaying. In the spayed females the weight of the hypophysis was only slightly greater than that of the controls, amounting to 3.84 per cent which should be considered as within the limits of experimental error and individual variation. The male rats however showed a striking difference in this respect. In the males which had been castrated the hypophysis was 73.62 per cent heavier than in the controls. This enlargement was shown in all of the four groups examined and in each litter. Along with this difference another was noticed, namely that in the females spaying was invariably followed by general overgrowth and obesity, while among the males castration was not followed by these changes. Hatai in a recent contribution (12) further substantiates these conclusions.

Stotsenburg (21) seems to have been the first to make a systematic study of the growth after castration of males among mammals, using the albino rat and concludes by saying "In the case of albino rats, the growth curve for castration is similar to that for normals." As a result of spaying females he reports in a later paper (22) an excess over normals in body length of 3.4 per cent and in body weight of 23.5 per cent and the excess in body length would call for, as he says, an excess in body weight of 12 per cent. Then the difference between 23.5 per cent and 12 per cent must be credited to the deposition of fat. This was confirmed at the post mortem examination. These results as already seen are substantiated by Hatai.

This relationship between the response of the hypophysis on the one hand and general overgrowth on the other has been noticed in the present investigation upon rabbits, the data for which had been recorded before the results of Hatai or Stotsenburg appeared, and will be considered later in this paper.

Some observers as already mentioned explain the hypertrophy of the pituitary following castration as an example of vicarious action. When the sexual glands are removed, the pituitary enlarges to supply some internal secretion to take the place of one which is lacking. Hoskins (14) is of the opinion that the pituitary is normally held in check by the gonads and when this inhibition is removed the pituitary manifests

increased activity leading to altered metabolism, and thus to an overgrowth of different parts of the body such as occurs both in acromegaly and after castration.

Rosalind Wulzen (25) mentions the evidence of previous workers which is somewhat contradictory regarding the effects on growth following the increase or decrease in amount of anterior lobe secretion which is supplied to different animals either from natural or artificial sources. The preponderance of the evidence cited, however, indicates that the pituitary body either injected or ingested is able to cause a diminution in rate of growth in young animals and that this is due to something more than emaciation has been shown by some who measured the long bones and found a decrease in their length. She also concludes from her own experiments that growth of young fowl is retarded by the addition to the diet of fresh, unmodified anterior lobe of ox pituitary. This was shown in body weight and in length of the long bones, and was more marked in the males than in the females.

MATERIAL

In the experiments here reported the rabbit has been chosen as the experimental animal for the reason that several other observers have used this form and have disagreed as to the results, also because it is inexpensive and at the same time easily obtained and cared for. In all, about 150 rabbits were obtained, of which several were lost in various ways. Since the fact that animals even of the same species raised in different sections of the country may possess marked difference, as is well shown by Marine (18) in case of the thyroids of dogs, it should be stated here that the animals used in this experiment were all born and raised in the neighborhood of Ithaca. Some were bought of farmers, while others were reared in the animal house belonging to the Physiological Department. All animals which seemed to be in any way abnormal were rejected. At the time of operation the animals varied in age from a few weeks to about one year. The body weights ranged from 300 grams to more than 2 kilos as will be seen in the accompanying tables. As to sex they were divided very nearly into two equal groups. Two series of animals have been used, the first including about 60 and the second about 90. The former was observed and examined during 1912 and the latter in the following year. Several objections, which might be raised, became apparent in case of the first series, namely, the animals were not necessarily of the same litter

although in many cases they probably were. A large proportion was full grown and hence possibly not so suitable for this experiment, and it was not certain that all the females were virgins. For these reasons it was thought advisable to repeat the work on a larger scale, making special effort to control, as far as possible, each operated animal by a normal one of the same litter, and to operate upon all at as early an age as practicable.

METHODS

Relative to the subject of castration and spaying a few general statements may be made. The effect upon the hypophysis following the removal of the testes or ovaries is a difficult question to solve, not because either operation is so complicated, but because of the small size of the hypophysis and its great normal variation as may be seen from the control group. Further difficulty is encountered by the fact that a greater normal variation is apparent when comparison is made between different litters, between animals from different sections of the country, between those which have and those which have not been pregnant, and between animals of different breeds. With these difficulties in mind, precaution has been taken to avoid to the greatest possible degree every source of error, compensating the unavoidable ones by the use of larger numbers of animals than would otherwise be necessary. By an examination of the literature in these particulars a noteworthy fact is revealed that in many experiments which are often quoted the above mentioned precautions were not taken. In some cases the conclusions are drawn from a few operated animals, with an even smaller number as controls, no mention being made as to sex, ratio of hypophysis to body weight, age, time elapsing after operation, or relationship of operated to control animals. These methods lead one to doubt the correctness of the conclusions to which many previous workers have come. Some, however, have worked with great care and this may explain in some respects why their results contradict those of others.

In the present investigation the animals which were purchased from the outside were placed in two pens in order to keep the sexes apart, the individual weight recorded, and a distinguishing number assigned. The color markings which do not change can also be relied upon as a guide to distinction. The litters of rabbits which were born here in our animal house were kept separate until large enough to be marked. They were then weighed as in the above case. Each animal to be operated on was selected whenever possible so that it could be controlled

by one of the same litter, of approximately the same weight at the time of operation, and always of the same sex. More attention was paid to these points in the second series than in the first.

In the case of young animals which were raised by us, the operation was performed as early as it was regarded advisable with a reasonable degree of safety. Those which were bought from other breeders were usually operated on as soon as received.

In all cases the operation was performed while the animal was under deep ether anesthesia, with aseptic precautions. In the case of the males the testes were removed through a short transverse incision through the skin of the public region. The cord was clamped, cut, and cauterized to obviate the use of a ligature. The ovaries were removed from females sometimes through a median longitudinal incision and sometimes through two opposite lateral incisions in the abdominal wall. After the wounds had been closed a 5 per cent solution of iodine in 70 per cent alcohol was applied occasionally for two or three days and no bandages were used. Only one or two fatalities could possibly be attributed to the operation. Sections of each piece of tissue removed were fixed for future histological examination.

The operated and control animals were kept in the same pen, always in the open air and under precisely the same conditions in every respect. All were well cared for and fed on such food as grass, vegetables, and apples. They were weighed individually on Saturday morning of each week before feeding, and from these weights curves of growth were plotted.

The animals were killed by coal gas. This is not only a convenient and rapid method but was also advantageous in this case because it delays clotting of the blood. The exact weight was now recorded, the bladder emptied by digital pressure and the body weight again taken. The head was now removed and the whole animal allowed to bleed freely, thus reducing the chance of error in weight of the hypophysis and other organs by inclusion of a varying amount of blood.

The next step and the one which required the most careful technique was the removal of the pituitary body. The points to be attended to in this dissection are the complete removal of its surrounding membranes, the severance of the pituitary from the infundibulum at the same point, and these without injury to the organ itself. Different methods of approach were tried on a few of the first series, but it was soon found that the ventral route was preferable. The head being severed the ventral part could be easily removed at the level of the

mouth by breaking the ramus of the mandible with bone forceps and then extending the angle of the mouth posteriorly by cutting the muscles and skin. The soft palate and mucosa covering the base of the sphenoid bone was then removed, and the position of the pituitary determined by locating the canalis craniopharyngeus which is directly ventral to the pituitary body. The base of the skull was now removed piecemeal by small bone forceps with care not to disturb the tissue in the region of the pituitary body; a small part of the sphenoid bone directly ventral to it being left until all the rest had been removed. When the pituitary is now exposed and the surrounding membranes carefully ruptured the gland will "shell out" when lifted ventrally and anteriorly from behind, from the tentorium sellae in much the same way as the brain can be lifted from the base of the skull from behind when the dura mater is removed from the dorsal surface, and the cranial nerves cut. As the pituitary is lifted out ventrally the infundibulum is flexed and breaks at the same place, in all cases near the pituitary at what is apparently a natural weak point in its structure. The entire body composed as Herring (13) describes of anterior and posterior lobes and pars intermedia, now free, was placed on a small watch glass and transferred immediately to a sensitive chemical balance, where its weight was determined to the tenth of a milligram. The next step in order for each animal was the removal, weighing and fixing of external parathyroids, thyroids, pancreas, spleen, suprarenals, kidneys, thymus, and ovaries if present, or if a male the prostate and also the testes if the animal is a control. Only a small piece of suitable size was placed in the fixing fluid, and the weights determined only for the thyroids, suprarenals, kidneys, testes, ovaries, and uterus. The same procedure was followed in all cases, the primary object being to secure data suitable for purposes of comparison, from both normal and operated animals.

As previously mentioned it was considered necessary to determine for each individual the reduced body-weight. This term will be used throughout to designate the live weight of the animal less the weight of the urine and gastro-intestinal contents, which is found by subtracting the latter from the weight taken immediately after the urine had been expelled. To determine the weight of the gastro-intestinal contents for this purpose, the whole tract from the cardia to the lower end of the rectum was removed with as little of the mesentery as possible and weighed. The contents were forced out by pressure along the outside with a piece of sheet cork which does not cut the tissue, and the

whole tract again weighed. This weight was then subtracted from the weight of the tract before contents were removed, thus giving the weight of the contents.

From the figures thus obtained the weight of the hypophysis in proportion to body-weight was calculated by dividing the weight of the hypophysis in milligrams by the reduced body-weight in kilos. The same method was followed in determining the relative weight of the other organs to body-weight, except in some instances where the weights are given in grams instead of milligrams.

RESULTS OF PRESENT INVESTIGATION

In presenting the results of this investigation the two series will be considered separately. For the first the averages of the body weights as taken each week are given in Tables I to IV for castrated and control males, and for spayed and control females respectively. Tables V and VI show a comparison of these operated and normal animals without regard to sex.

For the second series corresponding figures are given in Tables VII to XII. Among the animals of this series *ten* castrated males could be controlled by *eight* males of the same litters, and from the females *six* could be controlled by *five* of the same litters. The average weights of these are grouped in Tables XIII to XVI.

Curves I to XVI (figs. 1 to 8) are plotted from these averages and are intended to show relative rates of growth of each group.

The primary object of the experiment was to compare averages and individual weights of the pituitaries of operated and control animals of both sexes. When comparisons are made the weight per kilo of reduced body-weight will be used except where otherwise mentioned. These are to be found in their respective columns of Tables XVII to XXVIII. It will be best to follow at the same time the corresponding curves and notice their relation to pituitary weight. Observe that in general when the pituitary responded to the operation by an increase in size, the body-weight of that group did not respond, and vice versa.

To begin with the first series the male group (Tables XVII and XVIII) of *ten* castrated and *eight* controls give an average for the pituitary of 11.7 mg. and 10.2 mg. respectively which amounts to a difference of 14 per cent. Accompanying this the two growth curves (I and II, fig. 1) are shown to follow the same general course throughout a period of nineteen weeks.

For the females (Tables XXI and XXII) the spayed group of *twelve* show an average of 12.7 mg. as compared with 13.5 mg. for the *eleven* normals, or a difference of 6 per cent in favor of the latter. The curves (III and IV, fig. 2) show a remarkable difference in that the spayed animals increase in weight much faster than the normals. A part of this difference is evidently due to the fact that the normal animals were larger at the beginning of the experiment, but it scarcely appears reasonable to attribute the whole difference to this cause.

Taking the whole series (Tables XIX and XX) of *twenty-two* operated animals and *nineteen* controls without regard to sex the respective pituitary weights are 12.2 mg. and 12.1 mg., a difference of less than 1 per cent. The curves (V and VI, fig. 3) are resultants of those already mentioned and apparently show a gain in body-weight by the operated, accompanied by a negligible gain by the pituitary.

Consider now the second series and we find the male group (Tables XXIII and XXIV) composed of *twenty-one* castrated and *sixteen* controls with corresponding pituitary weights of 16.1 mg. and 14.6 mg. or a gain of 9 per cent by the castrated animals. With this gain in mind note that there is practically no overgrowth of the castrated animals in body-weight as shown by curves VII and VIII (fig. 4).

For the *nine* spayed females and *eleven* normals (Tables XXVII and XXVIII) the average pituitary weights are 16.1 mg. and 13.2 mg. respectively, making a difference of 23 per cent gain by the operated animals. Accompanying this marked gain the curves (IX and X, fig. 5) show that the spayed group not only did not gain over the normals but actually lost in comparison in body-weight.

When we group the second series without regard to sex (Tables XXV and XXVI) we have *thirty* operated animals with an average pituitary weight of 16.1 mg. and *twenty-seven* controls whose pituitaries average only 14.1 mg. showing a gain of 14 per cent after operation. The curves (XI and XII, fig. 6) by the same grouping show a striking similarity.

The grouping which should probably be considered the most conclusive is the one in which the experimental animals were each controlled by an animal of the same litter. The averages of these are given in Tables XXIII and XXIV for males and Tables XXVII and XXVIII for females and are marked thus *. *Ten* castrated males with an average pituitary weight of 15.2 mg. are in this way compared with *eight* controls with a corresponding weight of 15.6 mg. which is about 3 per cent in favor of the latter. The corresponding curves (XIII and XIV, fig. 7) do not show a marked increase by the castrated animals over the controls but toward the end of the period it may be noticed

that the castrated animals are about three weeks in advance of the normals which indicates a distinct gain.

In case of the females by this grouping (Tables XXVII and XXVIII) an increase of 25 per cent by the spayed is to be seen from 16.4 mg. and 13.1 mg. for the *six* spayed and *five* control animals respectively. A reverse is noticed in the curves (XV and XVI, fig. 8) from what is shown by the males in the two preceding curves and accompanying this a reverse in the pituitary weights as well.

These results shown in the several different groupings agree with the conclusions of Hatai for the albino rat in that when the body responds by an increase in weight even though slight the pituitary does not show a compensatory hypertrophy, while on the other hand, when the animals which were deprived of their sexual glands do not show an overgrowth there is a distinct, though in no case a very marked, increase in the weight of the pituitary. The rabbits in this case show more of a tendency on the part of the females to a hypophyseal hypertrophy, while in the albino rat according to Hatai the marked hypertrophy was noticed in the males alone.

The question may arise as to whether or not the hypertrophy might not be constant among those animals which do show an increase in body-weight as well as among those which do not, and that by the overgrowth of the body the hypertrophy of the former is not apparent when given in milligrams per kilo of reduced body-weight. An examination of the data however shows that this is not the case, for if the gross weights of the pituitaries be taken instead of the per kilo weights the results will be found to be practically the same.

The evidence set forth by former investigators show that in all probability a hypo-secretion of the pituitary produces an abnormal accumulation of fat, possibly due to lowered oxidation. According to Hatai (10) if a compensatory growth of the hypophysis does not follow, as in the case after spaying of albino rats, the secretion of the unchanged gland must be used for two purposes; first, to replace the ovarian hormone and second for the normal uses whatever these may be. Removal of the sex glands thus seems in some cases to overtax the normal pituitary with a result similar to that of hypo-secretion. In case of hypertrophy of the gland there seems to be no results, attributable to a decreased supply of the secretion, in the form of overgrowth or obesity. Semi-spaying in albino rats seems to produce further evidence in support of this view, since this operation produces neither a change in weight of hypophysis nor an increase in body-weight. The remaining ovary however has been stated to enlarge to twice its normal size and thus prob-

ably compensates for the lack of secretion from the one which was removed, consequently preventing the changes which would follow if this compensation had to be taken care of by the hypophysis. Be this as it may, it appears for the present to offer a reasonable explanation for the results obtained by comparing the growth curves with the corresponding pituitary averages.

Erdheim and Stumme (6) attribute the enlargement of the hands and lips which are sometimes observed in pregnancy to a hypersecretion of the pituitary. It seems reasonable to regard the hypertrophy during pregnancy as an extra stimulus to growth which is used in the development of the embryo, and that the persistence after parturition may serve as a galactagogue for the further increase in growth of the young after birth.

The difference in the manner in which the pituitary reacts to castration and spaying in different species and even in the same species as reported by different observers may possibly be explained otherwise than by assuming that some have been mistaken in their conclusions. The gland may in some cases be capable of taking on an additional amount of work, or at other times the generative glands might not be producing a secretion at the time of removal which would subsequently have to be produced by some other organ. Another explanation of the variations in results might lie in the possibility of accessory particles of the gland as reported by some as having been separated off along the path of development. These accessory portions may respond by an increase in activity, hence making it unnecessary for the pituitary itself to show any change. In still other species there may be no relation between the hypophysis and the generative glands. Some writers as already mentioned favor the theory that the generative organs have an inhibitory action on the pituitary and when these are removed the pituitary is freed from this inhibition, and consequently increases in size and activity.

In regard to the other organs which were examined the uterus may be mentioned first. In Tables XXVII and XXVIII the weights are shown to be 0.62 gram and 1.32 grams respectively for spayed and control females. One of the group, however (no. 64), was apparently in heat at the time she was killed, and this explains the unusually heavy weight of the uterus. Excluding this one the average for the control group is 0.95 gram thus making the uterus of the spayed rabbits average 35 per cent less than for the normals.

As to the heart, no effect can be said to follow castration or spaying. Attention should however be called to the averages given in Tables

XXIII and XXVIII which show that the smaller animals in general have a larger heart, in proportion to reduced body-weight, than the larger animals. This has also been mentioned in a previous report in case of the gastro-intestinal tract and gastro-intestinal contents (17).

The kidney weights, free from the capsules as given in Tables XXIII to XXVIII, are slightly less for operated than for control animals of both sexes. In case of the males this is in accord with what might be expected because the castrated animals are the heavier, but it does not hold true for the females since the normals are heavier. The difference however is probably too small considering the variation among individuals to justify any claim to an effect following castration or spaying.

Weights for the thyroids are given in Tables XXIII to XXVIII. For females the slight excess shown by the spayed animals is well within the limits of experimental error and individual variation. The whole group of normal males when compared with the whole group of castrated animals shows about 25 per cent in favor of the former, but when a comparison is made in like manner between those selected from the same litters this difference is reduced to less than 10 per cent. It is possible that this apparent reduction in weight of the thyroids is due to castration, but when we notice that among the normal males this organ varies from 52 grams to 91 grams we find ourselves in doubt about a definite conclusion.

The results obtained by previous investigators as to the effect on the suprarenal glands produced by castration and spaying in different animals is briefly summed up by Hatai (12). The results of different observers conflict, but most of them agree that the suprarenals and sexual glands are closely related and that an effect is produced on the former by castration and spaying. The females of the present experiment show no effect except by grouping animals of the same litters. In this manner a gain of 9 per cent is shown by the spayed animals. The whole series of castrated males show a gain of 20 per cent compared with normals, but by selecting those of the same litter for comparison this difference is reduced to only 6 per cent. By comparing the operated animals of the whole series with all the controls, without regard to sex, a gain of 14 per cent is shown by the operated animals. This organ also shows a high degree of variation which however is probably not enough to offset the apparent increase in size following the removal of the sexual glands especially in the case of the male rabbits.

The thymus gland, owing to the large amount of fat inseparably connected with it, was not weighed.

An attempt was made to weigh the pineal gland of each animal, but

on account of its small size and the difficulty of accurate dissection of fresh specimens only a few weights were taken.

The weights of testes and ovaries for normal animals are recorded in Tables XXIV and XXVIII respectively.

No histological examination of the tissue preserved has yet been made.

CONCLUSIONS

From the results of the experiments described, the following conclusions for the rabbit may be drawn.

1. There is no constant sex difference in the weight of the hypophysis.
2. Neither males nor females show a constant hypophyseal hypertrophy following castration or spaying.
3. The females may be regarded as showing a more constant response by the hypophysis after spaying than is to be seen among the males after castration.
4. From the curves of growth corresponding to each group there is a constant relationship between the rate of increase in body-weight and the response of the hypophysis to castration or spaying.
5. There is less hypertrophy of the hypophysis in those groups which show an increase in rate of growth.
6. In groups where no effect can be shown upon the rate of growth a distinct hypertrophy of the hypophysis is constant though in no case is it very marked.
7. A marked atrophy of the uterus follows removal of the ovaries from females.
8. No change in the weight of the heart or the kidneys can be attributed to castration or spaying.
9. No change can be demonstrated in the thyroid with the possible exception of a moderate decrease in males after castration.
10. The suprarenals show no marked effect. In the males a tendency toward enlargement follows castration, which does not appear after spaying females.
11. No conclusions were reached as to the effect of castration or spaying on the thymus, or pineal gland.

It is with a feeling of true gratefulness that I wish to express in conclusion my thanks to Prof. Sutherland Simpson for providing the material with which this investigation has been carried out, for his many valuable suggestions, and encouragement at all times.

SERIES NO. I		TABLE I	TABLE II	TABLE III	TABLE IV	TABLE V	TABLE VI
		AVERAGE WEIGHT OF CASTRATED MALES	AVERAGE WEIGHT OF CONTROL MALES	AVERAGE WEIGHT OF SPAYED FEMALES	AVERAGE WEIGHT OF CONTROL FEMALES	AVERAGE WEIGHT OF OPERATED MALES AND FEMALES	AVERAGE WEIGHT OF CONTROL MALES AND FEMALES
Number of animals		10	8	12	11	22	19
May	18	1.295	1.312	0.911	1.560	1.127	1.380
	25	1.355	1.359	0.984	1.583	1.199	1.416
June	1	1.378	1.359	0.992	1.536	1.210	1.404
	8	1.411	1.433	1.031	1.559	1.245	1.463
	15	1.454	1.489	1.076	1.587	1.301	1.489
	22	1.498	1.563	1.128	1.620	1.377	1.543
	29	1.563	1.613	1.159	1.647	1.398	1.592
July	6	1.628	1.666	1.199	1.701	1.448	1.639
	13	1.678	1.703	1.246	1.717	1.499	1.670
	20	1.739	1.745	1.295	1.732	1.555	1.689
	27	1.896	1.826	1.471	1.835	1.728	1.779
August	3	1.942	1.863	1.550	1.868	1.794	1.820
	10	1.999	1.918	1.679	1.905	1.878	1.870
	17	1.999	1.941	1.699	1.894	1.865	1.873
	24	2.014	1.984	1.753	1.895	1.930	1.916
	31	2.132	2.083	1.925	2.017	2.069	2.026
September	7	2.199	2.190	2.018	2.060	2.165	2.099
	14	2.241	2.199	2.095	2.105	2.214	2.126
	21	2.318	2.240	2.199	2.169	2.296	2.180
	28	2.378	2.234	2.266		2.362	2.216

SERIES NO. II	TABLE VII AVERAGE WEIGHT OF CASTRATED MALES	TABLE VIII AVERAGE WEIGHT OF CONTROL MALES	TABLE IX AVERAGE WEIGHT OF SPAYED FEMALES	TABLE X AVERAGE WEIGHT OF CONTROL FEMALES	TABLE XI AVERAGE WEIGHT OF OPERATED MALES AND FEMALES	TABLE XII AVERAGE WEIGHT OF CONTROL MALES AND FEMALES	TABLE XIII AVERAGE WEIGHT OF CASTRATED MALES	TABLE XIV AVERAGE WEIGHT OF CONTROL MALES	TABLE XV AVERAGE WEIGHT OF SPAYED FEMALES	TABLE XVI AVERAGE WEIGHT OF CONTROL FEMALES
Number of animals.....	21	16	9	11	30	27	10	8	6	5
Weight when operated...	1.161	1.181	1.177	1.049	1.166	1.133	0.887	0.869	0.886	0.865
June 28.....	1.183	1.202	1.123	1.064	1.165	1.146	0.910	0.889	0.833	0.901
July 5.....	1.278	1.316	1.194	1.123	1.253	1.232	1.055	1.031	0.885	0.999
12.....	1.379	1.420	1.302	1.300	1.356	1.371	1.137	1.056	0.993	1.148
19.....	1.385	1.442	1.332	1.348	1.376	1.404	1.167	1.084	1.086	1.206
26.....	1.402	1.477	1.409	1.428	1.404	1.458	1.226	1.121	1.133	1.311
August 2.....	1.484	1.519	1.468	1.520	1.479	1.519	1.319	1.232	1.190	1.390
9.....	1.539	1.588	1.551	1.615	1.542	1.598	1.377	1.320	1.273	1.487
16.....	1.635	1.642	1.614	1.691	1.628	1.662	1.482	1.346	1.367	1.561
23.....	1.701	1.700	1.670	1.768	1.692	1.727	1.572	1.415	1.492	1.639
30.....	1.764	1.754	1.718	1.864	1.750	1.799	1.649	1.474	1.500	1.725
September 6.....	1.792	1.766	1.792	1.903	1.792	1.821	1.664	1.508	1.577	1.735
13.....	1.835	1.787	1.835	1.986	1.834	1.868	1.702	1.529	1.640	1.831
20.....	1.875	1.812	1.883	2.032	1.877	1.901	1.779	1.585	1.688	1.849
27.....	1.948	1.875	1.914	2.027	1.937	1.940	1.868	1.676	1.726	1.830
October 4.....							1.897	1.758		
11.....							1.961	1.811		

TABLE XVII

TABLE XVII

SERIES NO. 1	NUMBER OF ANIMAL	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT IN MILLIGRAMS OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF THYROIDS IN MGS. PER KILO OF R. B. W.
Castrated Males	3	155	0.425	2.300	2.285	112.0	324.0	1.943	23.0	11.837	75.14
	17	157		2.345	2.345	131.0	379.0	2.066	35.0	16.940	
	19	158		2.390	2.390	140.0	180.0	2.210	32.0	14.479	56.56
	13	148	2.125	2.530	2.515	118.0	239.0	2.276	27.0	11.862	46.56
	11	185	0.975	2.675	2.640	186.0	340.0	2.300	40.0	17.391	
	1	45	3.375	3.000	3.000	176.0	409.0	2.591	15.0	5.789	59.17
	15	208	1.050	2.885	2.885	154.0	270.0	2.615	27.0	10.325	
	5	199	0.875	3.010	3.010	180.0	315.0	2.695	30.0	11.131	
	9	189	0.860	3.165	3.160	188.0	250.0	2.910	22.0	7.560	70.10
	7	187	0.800	3.650	3.650	195.0	312.0	3.338	33.0	9.886	69.50
Average			1.311	2.795	2.788	158.0	293.0	2.495	28.0	11.710	61.31

TABLE XVIII

Control Males	4			1.700	1.650	114.0	134.0	1.516	14.0	12.25	71.89
	2			2.490	2.490	140.0	515.0	1.976	22.0	11.19	
	10			2.390	2.390	144.0	276.0	2.114	20.0	9.41	
	6			2.350	2.350	120.0	226.0	2.124	20.0	9.41	53.69
	12			2.450	2.425	128.0	268.0	2.157	20.0	9.27	38.48
	8			2.745	2.725	140.0	150.0	2.575	27.0	10.48	70.29
	16			3.110	3.090	162.0	330.0	2.760	31.0	11.23	63.76
	14			3.350	3.325	144.0	316.0	3.009	26.0	8.64	62.14
Average				2.573	2.555	137.0	277.0	2.279	23.0	10.24	60.21

TABLE XIX

Average for operated males and females ...	2.632	2.625	151.0	284.0	2.344	29.0	12.26	58.13

TABLE XX

Average for control males and females ...	2.396	2.387	135.0	253.0	2.112	26.0	12.12	62.54

TABLE XXI

SERIES NO. 1	NUMBER OF ANIMAL	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B.$_w$W.) IN KILOS	WEIGHT IN MILLIGRAM OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF THYROIDS IN MGS. PER KILO OF R. B. W.
	35	68	0.450	0.935	0.935	71.0	146.0	0.789	7.0	8.87	
	23	85	1.350	1.810	1.810	108.0	262.0	1.548	27.0	17.44	
	33	156	0.480	1.890	1.880	135.0	256.0	1.684	23.0	13.66	89.66
	39	89	2.250	2.350	2.340	140.0	364.0	1.976	32.0	16.18	34.41
	27	179	0.465	2.525	2.525	158.0	302.0	2.223	25.0	11.24	32.84
Spayed	29	181	0.920	2.800	2.778	165.0	325.0	2.453	25.0	10.18	
Females	25	175	1.140	2.695	2.675	150.0	215.0	2.460	28.0	11.38	
	43	87	2.170	2.770	2.770	143.0	299.0	2.471	34.0	13.76	33.91
	37	178	0.765	2.875	2.860	184.0	306.0	2.554	28.0	10.96	67.34
	41	26	2.990	3.130	3.130	160.0	335.0	2.795	59.0	21.11	
	21	189	1.500	3.100	3.090	140.0	293.0	2.797	33.0	11.08	55.06
	31	178	0.970	3.075	3.075	180.0	214.0	2.861	20.0	6.99	74.45
Average.....		133	1.287	2.496	2.489	144.0	276.0	2.218	29.0	12.74	55.38

TABLE XXII

	32			0.890	0.890	60.0	134.0	0.756	5.0	6.65	
	26			1.725	1.725	122.0	198.0	1.527	31.0	20.30	
	30			1.950	1.950	140.0	272.0	1.678	14.0	8.34	78.07
	42			2.250	2.250	110.0	222.0	2.028	29.0	14.30	
Control	28			2.415	2.410	160.0	280.0	2.130	21.0	9.85	74.65
Females	38			2.515	2.490	149.0	323.0	2.167	35.0	16.10	66.91
	22			2.635	2.635	126.0	369.0	2.266	25.0	11.03	62.66
	34			2.560	2.550	160.0	250.0	2.300	30.0	13.04	53.48
	40			2.525	2.525	133.0	221.0	2.304	28.0	12.58	46.44
	24			2.575	2.570	156.0	226.0	2.344	29.0	12.37	69.54
	36			2.910	2.910	150.0	395.0	2.515	60.0	23.85	
Average.....				2.268	2.264	133.0	255.0	2.001	28.0	13.50	64.53

TABLE XXIII

SERIES NO. II	NO. OF ANIMAL	LITTER	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT IN MILLIGRAMS OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH THYROIDS IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH ADRENALS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH KIDNEYS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF HEART IN GRAMS PER KILO OF R. B. W.
	1		105	1.700	2.200	2.200	120.0	284.0	1.916	27.0	14.091	58.5	0.210	7.270	2.66
	3		106	1.975	2.450	2.450	112.0	180.0	2.270	26.0	11.894	55.5	0.180	6.787	2.48
	5		107	2.200	2.615	2.605	117.0	301.0	2.304	29.1	12.630	49.5	0.159	6.303	2.42
	7		100	1.925	2.050	2.050	114.0	174.0	1.876	30.0	15.943	60.8	0.406	6.215	2.84
	9		108	1.600	2.170	2.160	108.0	244.0	1.916	29.0	15.135	50.6	0.359	5.930	2.95
	11		103	1.310	1.750	1.740	96.0	168.0	1.572	24.6	15.666	52.8	0.237	5.203	2.74
	13		103	1.650	1.475	2.470	132.0	278.0	2.192	29.0	13.270	75.3	0.178	7.303	2.83
	15		101	1.725	2.225	2.225	117.0	232.0	1.993	29.0	14.601	43.7	0.296	6.126	2.56
	17	A*	112	1.275	2.400	2.400	152.0	272.0	2.128	30.6	14.379	52.2	0.337	5.430	3.05
	19	A*	112	1.275	2.500	2.475	144.0	280.0	2.195	32.0	14.578	60.1	0.222	6.300	3.05
Castrated	21	B*	112	1.025	2.170	2.160	106.0	322.0	1.838	23.0	12.513	62.0	0.114	8.890	3.18
Males	23	B*	112	0.800	1.825	1.800	118.0	176.0	1.642	24.8	15.364	70.2	0.168	7.881	3.17
	27	B*	112	0.850	1.750	1.740	102.0	270.0	1.470	23.0	15.646	54.4	0.170	8.823	3.22
	29	A*	127	0.900	2.540	2.525	141.0	383.0	2.142	35.4	16.526	57.4	0.307	8.235	3.03
	33	C	127	0.480	1.800	1.809	106.0	239.0	1.561	33.0	21.140	83.3	0.295	9.122	3.47
	35	C	127	0.430	2.200	2.200	120.0	380.0	1.820	44.6	24.505	61.5	0.238	8.247	3.23
	37	D	127	0.525	1.320	1.300	84.0	158.0	1.142	31.4	27.496	79.7	0.688	10.998	3.71
	41	E*	102	0.890	1.615	1.615	90.0	256.0	1.359	28.0	20.603	88.3			
	45	E*	123	0.855	2.050	2.050	115.0	185.0	1.865	26.0	13.941	62.2	0.165	7.501	3.03
	46	E*	125	0.625	2.220	2.210	122.0	281.0	1.929	24.6	12.752	50.8	0.140	6.526	2.96
	47	F*	125	0.375	1.725	1.710	117.0	273.0	1.437	23.0	16.005	66.1	0.177	7.702	3.05
Average				1.161	2.049	2.090	116.0	254.0	1.836	28.7	16.127	61.6	0.252	7.339	2.98
Average*			116	0.887	2.182	2.169	120.7	269.8	1.801	27.0	15.231	62.4	0.200	7.476	3.08

TABLE XXIV

SERIES NO. II	NO. OF ANIMAL	LITTER	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT OF PITUITARY IN MILLIGRAMS	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH THYROIDS IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH ADRENALS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH TESTES IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH KIDNEYS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF HEART IN GRAMS PER KILO OF R. B. W.
	2			1.700	2.050	2.042	128.0	207.0	1.835	26.5	14.441	61.5	0.193	1.65	7.37	2.89
	4			1.825	2.135	2.135	115.0	308.0	1.827	23.8	13.015	74.4	0.235	1.22	8.99	2.91
	6			2.175	2.400	2.380	102.0	171.0	2.209	23.6	10.683	64.2	0.161	2.68	7.57	2.62
	10			1.550	1.860	1.820	95.0	153.0	1.667	22.2	13.317	57.5	0.252	2.13	8.02	3.51
	12			1.575	1.790	1.700	82.0	121.0	1.579	26.8	16.972	77.2	0.329	1.86	6.72	2.72
	16			1.850	2.075	2.075	112.0	270.0	1.805	24.0	13.296	59.2	0.282	1.22	6.37	2.74
	18	A*		0.900	1.830	1.830	104.0	198.0	1.632	20.0	12.254	64.9	0.333	3.05	9.55	3.62
Control	20	A*		1.350	2.510	2.453	123.0	301.0	2.151	28.6	13.296	52.4	0.173	2.41	7.00	3.35
Males	22	B*		1.050	2.230	2.230	112.0	262.0	1.968	30.8	15.650	58.9	0.129		8.42	3.23
	24	B*		0.740	1.530	1.530	100.0	188.0	1.337	19.6	14.659	65.0	0.187		7.91	3.59
	26	B*		0.675	1.820	1.800	120.0	298.0	1.502	29.6	19.707	53.9	0.167	2.24	7.88	4.04
	30			0.900	2.425	2.375	137.0	263.0	2.112	29.0	13.731	79.0	0.204	2.41	7.39	3.11
	42	E*		0.805	1.950	1.930	120.0	278.0	1.652	25.0	15.133	72.0	0.094	2.81	8.56	3.16
	44	E*		0.920	2.060	2.050	108.0	267.0	1.783	28.8	16.208	87.4	0.196	1.05	9.63	2.97
	48	F*		0.515	1.625	1.590	103.0	262.0	1.328	24.8	18.674	91.8	0.221	1.87	8.75	2.91
	51				2.150	2.140	120.0	328.0	1.812	25.4	14.017	65.7	0.209	3.52	10.63	3.09
Average				1.235	2.028	2.004	111.0	242.0	1.762	25.5	14.690	76.8	0.210	2.15	8.17	3.15
Average*				0.870	1.944	1.926	111.0	257.0	1.669	25.9	15.698	68.3	0.188	1.68	8.61	3.36

TABLE XXV

Average for operated males and females	2.052	2.074	117.0	245.0	2.499	28.5	16.137	61.5	0.242		7.23	2.94

TABLE XXVI

Average for control males and females	2.114	2.103	114.0	248.0	1.856	25.7	14.117	64.3	0.212		7.97	3.05

TABLE XXVII

TABLE XXVII

SERIES NO. II	NUMBER OF ANIMAL	LITTER	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT OF PITUITARY IN MILLIGRAMS	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH THYROIDS IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH ADRENALS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH OVARIES IN GRAMS PER KILO OF R. B. W.	WEIGHT OF UTERUS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH KIDNEYS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF HEART IN GRAMS PER KILO OF R. B. W.
	83	H*	116	0.855	2.075	2.075	130.0	173.0	1.902	22.0	11.56	62.0	0.201		1.23	5.91	2.80
	85	H*	116	0.920	2.120	2.090	124.0	214.0	1.876	23.2	12.36	53.8	0.202		1.58	6.25	2.69
	61		130	1.420	1.955	1.950	98.0	172.0	1.778	26.0	14.62	38.8	0.217		0.63	6.96	3.00
Spayed	65		123	2.125	2.285	2.280	129.0	262.0	2.018	36.2	17.94	53.0	0.279		0.93	6.25	2.88
Females	67		125	1.730	3.020	3.020	152.0	220.0	2.800	39.2	14.00	69.7	0.165		0.41	5.99	2.36
	69	A*	99	0.860	1.330	1.325	123.0	280.0	1.045	26.0	24.88	90.9	0.330		0.14	7.30	3.66
	71	B*	122	1.100	2.500	2.475	126.0	256.0	2.219	31.0	13.97	47.3	0.129		0.39	7.12	2.70
	73	B*	121	0.750	1.970	1.965	118.0	292.0	1.673	26.2	15.66	58.6	0.179		0.17	9.22	3.10
	75	G*	96	0.830	1.275	1.260	75.0	135.0	1.125	23.0	20.44	77.3	0.266		0.15	7.92	3.25
Average					2.059	2.049	119.0	223.0	1.826	28.1	16.16	61.3	0.218		0.62	6.99	2.94
Average*				0.886	1.878	1.865	116.0	225.0	1.643	25.2	16.48	64.9	0.218		0.61	7.29	3.04

TABLE XXVIII

	84	H*			2.050	2.050	130.0	212.0	1.838	18.0	9.79	64.4	0.219	0.038	0.79	6.16	2.46
	79	C			2.300	2.300	126.0	304.0	1.996	29.0	14.53		0.264	0.071		10.06	3.17
	80	C			2.225	2.225	120.0	294.0	1.931	26.2	13.57	69.9	0.328	0.078	0.91	8.41	3.28
	81	C			2.475	2.475	126.0	364.0	2.111	34.0	16.11		0.250	0.079	1.28	8.40	2.92
Control	62				1.930	1.930	88.0	155.0	1.775	25.8	14.54	62.5	0.280	0.101	1.48	6.56	2.81
Females	64				2.590	2.590	110.0	192.0	2.398	31.0	12.93	55.8	0.147	0.218	4.70	0.40	3.12
	66				3.220	3.220	123.0	187.0	3.033	26.0	8.57	46.4	0.130	0.113	1.30	5.66	1.94
	70	A*			2.190	2.180	138.0	382.0	1.798	31.0	17.24	77.8	0.238	0.061	0.77	8.64	3.18
	72	B*			2.455	2.450	132.0	296.0	2.154	24.0	11.14	55.2	0.111	0.058	1.06	8.29	2.87
	74	B*			1.900	1.890	104.0	248.0	1.642	24.0	14.62	37.1	0.174		0.29	6.91	3.01
	76	G*			1.405	1.405	90.0	180.0	1.225	16.0	13.06	54.6	0.257	0.101	0.66	7.63	2.95
Average					2.249	2.246	117.0	255.0	1.991	25.9	13.28	58.2	0.218	0.092	1.32	7.56	2.88
Average*					2.000	1.995	119.0	264.0	1.751	23.0	13.17	57.8	0.200	0.065	0.71	7.53	2.89

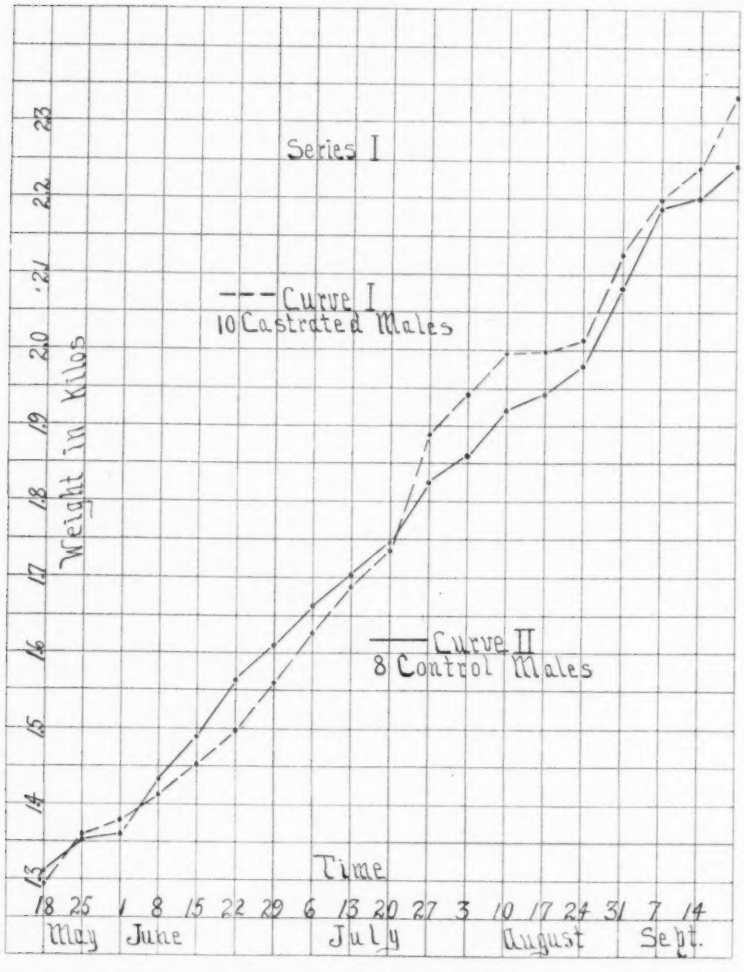

Fig. 1. Curve I (broken line), growth of ten castrated males. Series I. Average pituitary weight per kilo of R. B. W. 11.7 mg.

Curve II (solid line), growth of eight control males. Series I. Average pituitary weight per kilo of R. B. W. 10.2 mg. Difference, 14 per cent.

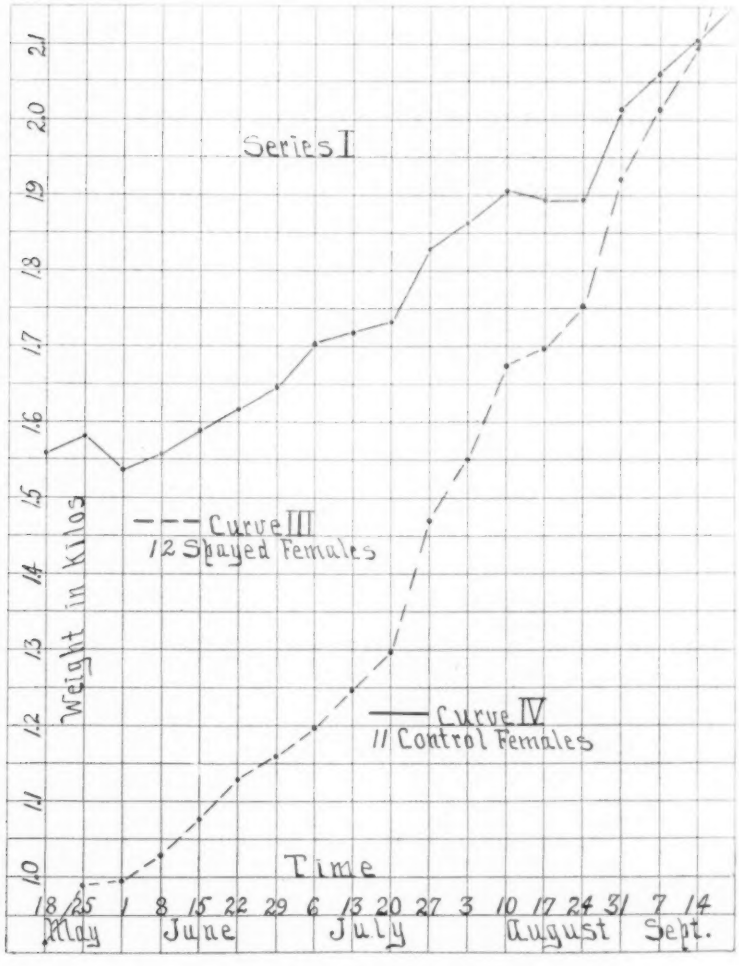

Fig. 2. Curve III (broken line), growth of twelve spayed females. Series I. Average pituitary weight per kilo of R. B. W. 12.7 mg.

Curve IV (solid line), growth of eleven control females. Series I. Average pituitary weight per kilo of R. B. W. 13.5 mg. Difference, 6 per cent.

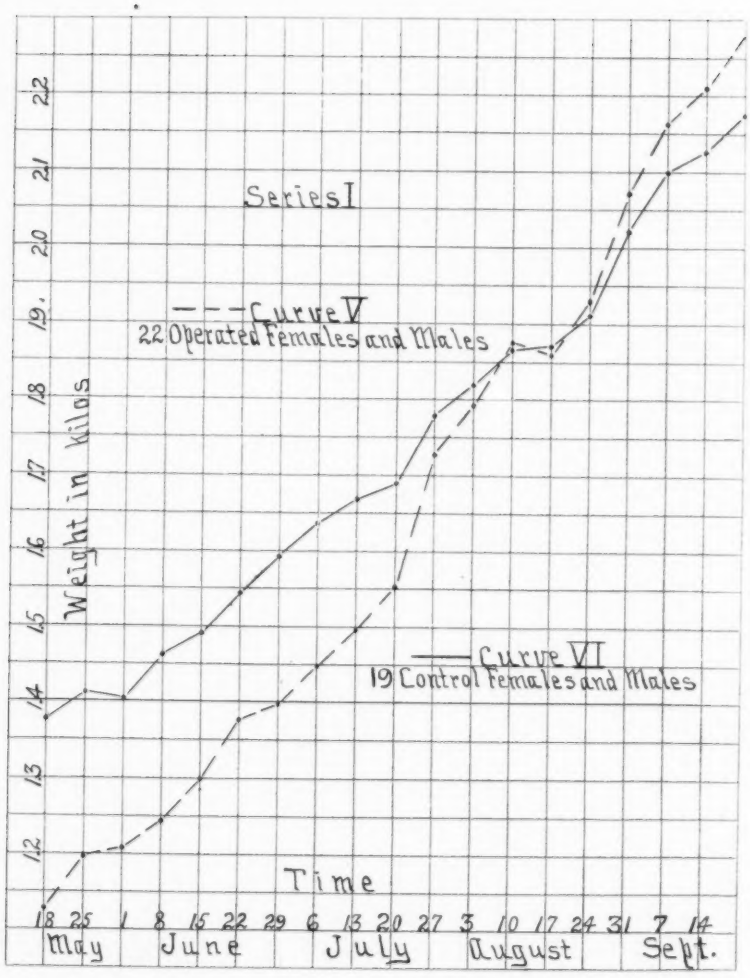

Fig. 3. Curve V (broken line), growth of twenty-two operated animals of both sexes. Series I. Average pituitary weight per kilo of R. B. W. 12.2 mg.

Curve VI (solid line), growth of nineteen controls of both sexes. Series I. Average pituitary weight per kilo of R. B. W. 12.2 mg.

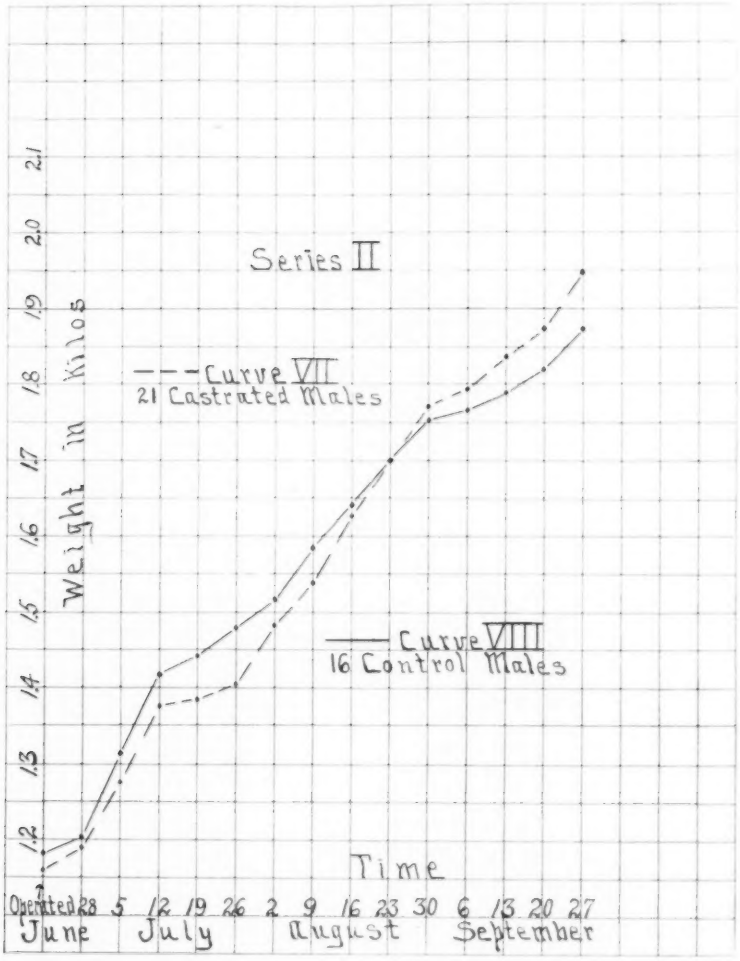

Fig. 4. Curve VII (broken line), growth of twenty-one castrated males. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve VIII (solid line), growth of sixteen control males. Series II. Average pituitary weight per kilo of R. B. W. 14.6 mg. Difference 9 per cent.

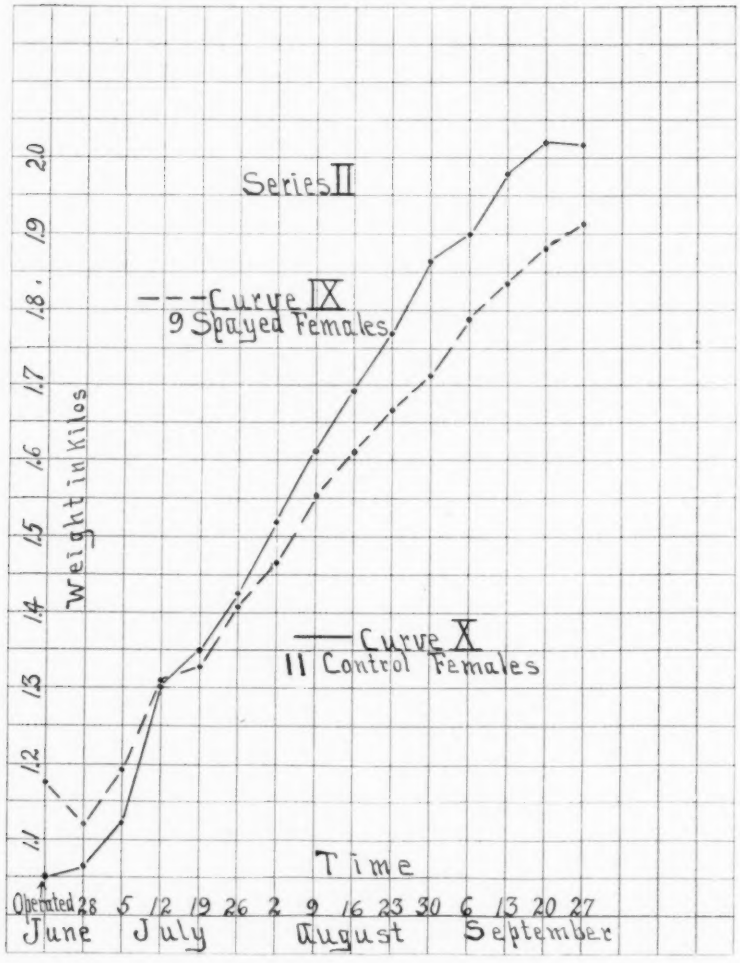

Fig. 5. Curve IX (broken line), growth of nine spayed females. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve X (solid line), growth of eleven control females. Series II. Average pituitary weight per kilo of R. B. W. 13.2 mg. Difference, 23 per cent.

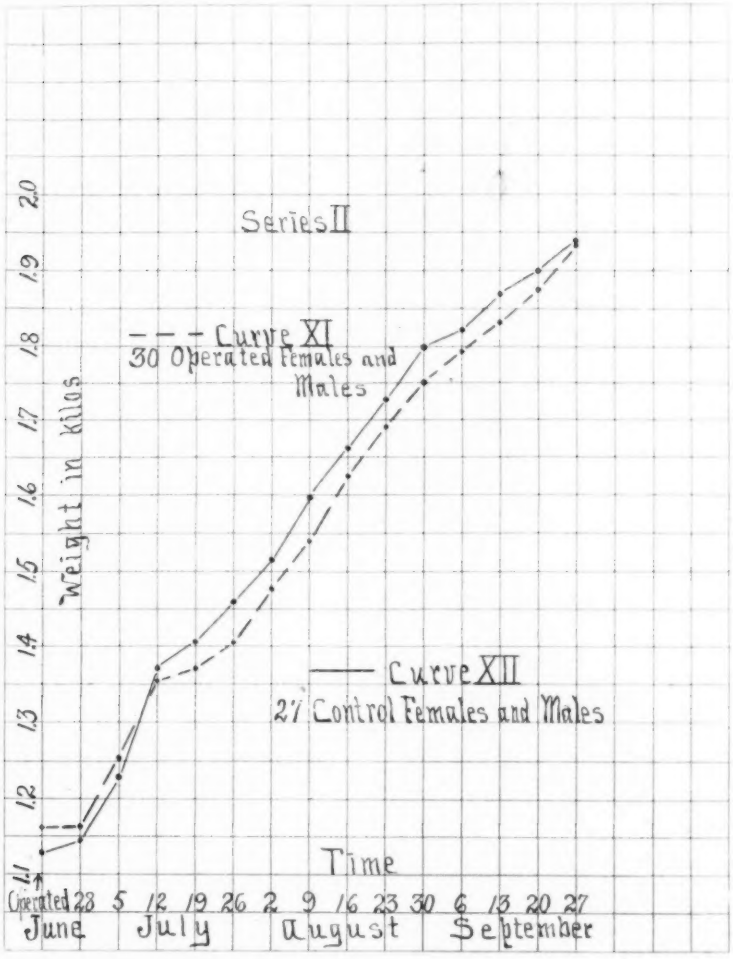

Fig. 6. Curve XI (broken line), growth of thirty animals of both sexes. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve XII (solid line), growth of twenty-seven controls of both sexes. Series II. Average pituitary weight per kilo of R. B. W. 14.1 mg. Difference, 14 per cent.

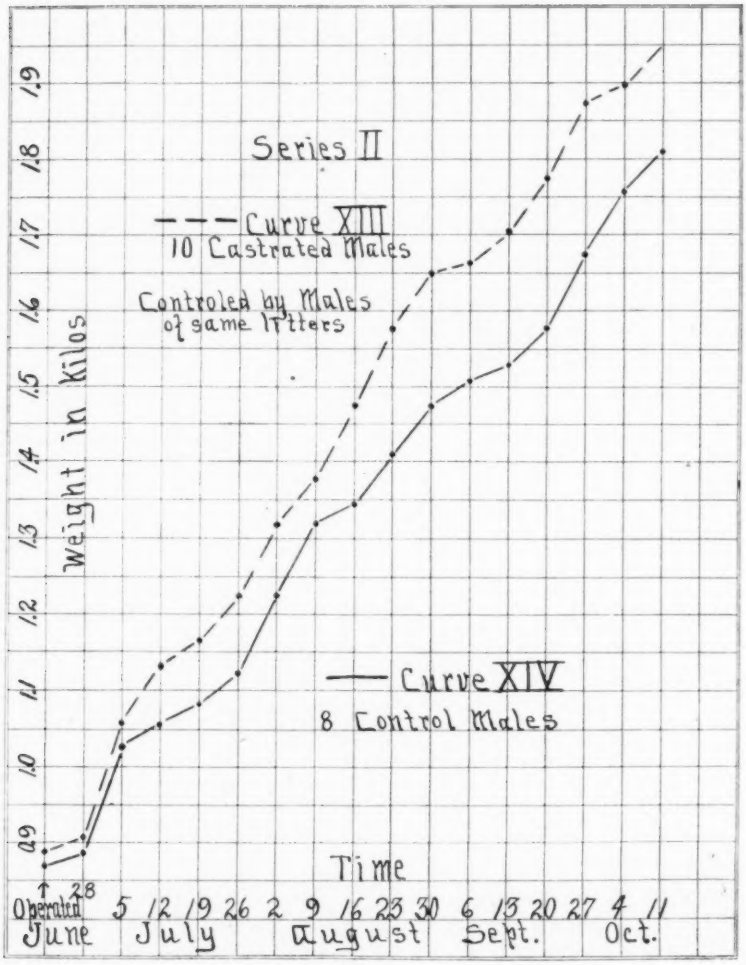

Fig. 7. Curve XIII (broken line), growth of ten castrated males controlled by animals of the same litters. Series II. Average pituitary weight per kilo of R. B. W. 15.2 mg.

Curve XIV (solid line), growth of the eight control males. Series II. Average pituitary weight per kilo of R. B. W. 15.6 mg. Difference, 3 per cent.

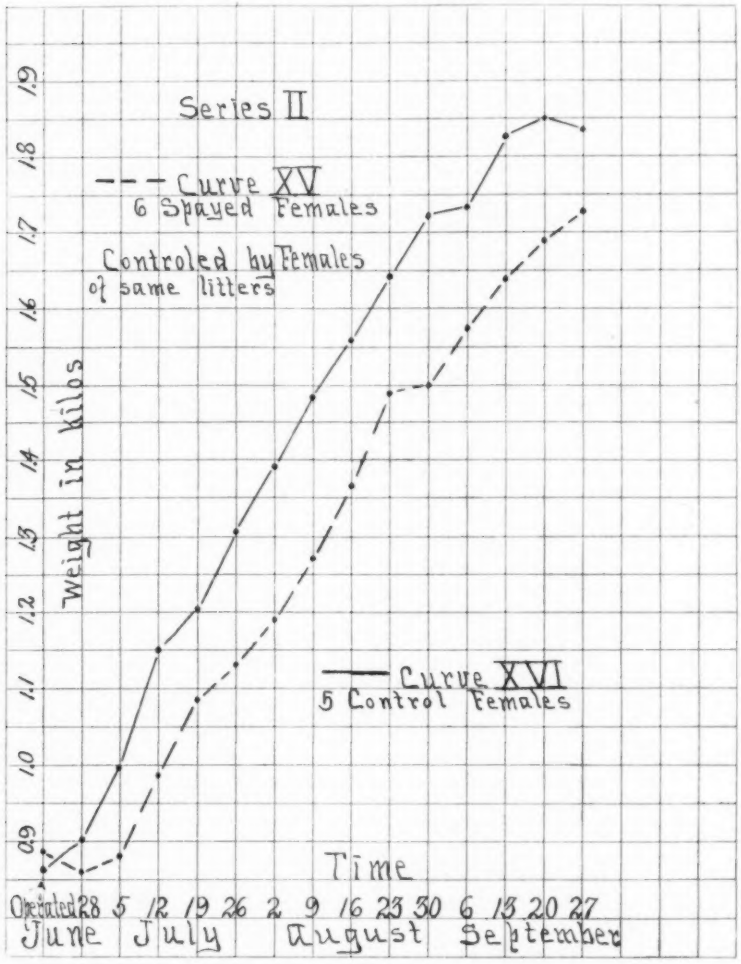

Fig. 8. Curve XV (broken line), growth of six spayed females controlled by animals of the same litters. Series II. Average pituitary weight per kilo of R. B. W. 16.4 mg.

Curve XVI (solid line), growth of the five control females. Series II. Average pituitary weight per kilo of R. B. W. 13.1 mg. Difference, 25 per cent.

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FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

VIII. The Influence of Certain Metals and the Electric Current

N. S. STERN

From the Laboratory of Physiology in the Harvard Medical School

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The earliest work on the effect of galvanism on the blood was done as far back as 1824 by Scudamore (1) who distinguished clearly between the effects at the two poles.

> Healthy blood was portioned into two cupping glasses. . . . Thirty pair of plates, four inches square, were employed for the galvanic action. Immediately on introducing the wires, at the negative one a mottled scum appeared, having in color, shades of green, red, and yellow, with a copious disengagement of gas. To the positive wire was attached a dense black coagulum which, as it dried, assumed the appearance of charcoal. . . . In three minutes the galvanized blood afforded a pellicle, when the other portion had not begun to coagulate. A similar difference continued; and at the expiration of nine minutes, the galvanized blood was very much advanced in coagulation, and the other gave only a dense coagulum from the bottom.

Eight years later Benjamin Phillips (2) published his findings on the earliest application of electricity to blood in circulation. In 1867 Duncan and Fraser (3) reported on work done up to that time, and further gave original cases and experiments on electrolysis in aneurism. Somewhat previously Steinlein (4), working with egg white found no change when platinum electrodes and a weak current were used, but found a coagulation at the positive pole when electrodes of oxidizable metals—tin, iron, copper, zinc—were used. Recently Hunner (5) has summarized the cases of aneurism treated by wire and galvanism up to 1900.

During some preliminary experiments we became aware of the remarkable effect of trivalent atoms on the precipitation of egg white and certain blood corpuscles. Mines (6) found that egg white is at once precipitated by a simple trivalent ion such as lanthanum (p. 211), and that the blood corpuscles of Scyllium canicula are at once agglutinated by

cerium chloride in a solution of 0.0008 molar, though some agglutination is present with a dilution of one-tenth of this (pp. 226, 227). Much higher concentrations of bivalent magnesium and univalent sodium are necessary to produce any agglutination. Consequently we determined to make use of wire of aluminum, a trivalent metal, and to compare it with copper, previously used in coagulation experiments by Cannon, and with iron originally used in wiring aneurisms.

The object of this study then was to determine quantitatively the effect of the three metals on the coagulation time of blood, and to determine the effect of the electric current as it passed into and through blood from the wire as a positive pole.

The method. The method was that devised by Cannon and Mendenhall (7) modified to suit the requirements of the experiment. The recording device consisted of a lever whose long arm wrote on a slowly moving kymograph directly above an electromagnetic time signal. On the short arm of the lever hung the wire which dipped into the blood. The lever was so balanced that the short arm plus the copper wire exceeded the long arm by about 30 mgm. Special weights were used when the aluminum and iron wires (of the same gauge) were employed so that the short arm still exceeded the long arm by 30 mgm. Another lever of sufficient weight rested across the long arm to prevent its rising except at the proper moment, and this second lever was lifted by a simple pulley device, and checked by an equally simple device. This modification was suggested in part by Mendenhall.

Preliminary experiments showed the evolution of gas at the negative pole as spoken of by Scudamore. To separate the poles so that the phenomena would be distinct, an addition was made to the tube and cannula employed by Cannon and Mendenhall. This addition consisted of a short rubber connection which joined the small end of the original cannula to one end of a short U made of the same glass as the cannulae, then another longer rubber connection which joined the other end of the U to a second cannula. The blood was drawn through the second cannula and was permitted to flow through the tubes until the proper amount was present in the original cannula. If a current was to be used, the second cannula was removed to allow easy escape of the gas evolved at the negative electrode. This negative electrode was a simple steel needle soldered to copper wire, and was passed through the second rubber connection just above the glass of the U-tube.

Electricity was drawn from a direct-current lighting circuit, and the amount flowing was easily controlled by a rheostat. From the positive post of the rheostat, the current flowed through a voltmeter, cali-

brated to read also under the conditions of the experiment in milliamperes. Thence the current passed through a pole changer, to the fulcrum of the recording lever, through the lever itself to the wire at its short end. This contact—lever to wire—was necessarily a loose one, and it was improved at first by amalgamation. This, while satisfactory for the copper, was not for the aluminum, but later the contact was satisfactorily established by the presence of a drop of Ringer's solution. The current then flowed through the wire into the blood in the original cannula, through blood in the constricted neck of the cannula (where considerable resistance was offered), through the blood in the tube to the negative electrode previously described. From here the course was through the pole changer to the negative post on the rheostat. In all the results used in this paper, if current was flowing at all, it was constant—kept so by manipulation of the rheostat if necessary.

Belgian hares, all of the same stock, were used because the coagulation time of their blood was known to be longer than that of cats. They were anæsthetized by urethane, 2.5 grams per kilo of body weight. Blood was drawn from the right femoral artery, just below the deep femoral branch. Other details, as of temperature, were carried out according to the directions of Cannon and Mendenhall.

For the first half minute, required to draw the blood and prepare the apparatus, the conditions were nearly constant. Consequently for the more accurate comparison of the effect of the various factors this half minute was subtracted in each case from the actual coagulation time. This allowed the first record in each case to be taken to prove that conditions were satisfactory. If current was used it was turned on as soon as possible after this test record—practically coincidentally.

Experimental results. The figures given are only those where the technique was unquestioned, and come from the nineteen last experiments of the series. Practically as many more earlier preliminary experiments were performed.

The first figures are arranged to show a comparison between copper, aluminum and iron, when they were used in the ordinary way without the passage of current.

METAL	NUMBER OF OBSERVATIONS	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
		minutes	*minutes*	*minutes*
Copper	44	9.6	16.5	5.0
Aluminum	32	5.3	9.5	2.0
Iron	14	8.3	13.5	5.5

In only two observations did the coagulation take longer with aluminum than with copper in the same blood.

In only two experiments of the nineteen was the average coagulation time with aluminum as great as with copper. In all other experiments the average clotting time with copper was definitely and consistently greater. Iron seemed to act rather like copper than like aluminum.

The average coagulation time with aluminum (5.3 minutes) was reduced from the average with copper (9.6 minutes), when no current was passing, by 45 per cent.

The use of electricity. The rheostat supplied between 35 and 40 volts. Owing to the high resistance (largely due to the constriction

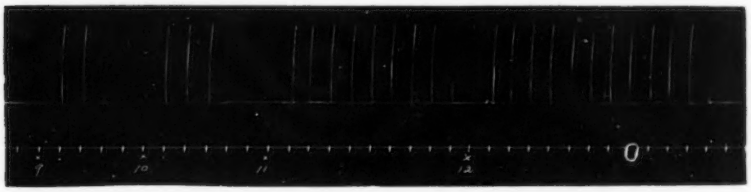

Fig. 1. Experiment 32. Factors affecting coagulation time of blood. The influence of certain metals and the electric current. A series of coagulation times under different conditions. *x*, Blood drawn. . Current on. Time in half minutes. *9*, Aluminum wire, 1 milliampere, time 1.5 minutes. *10*, Copper wire 1 milliampere, time 2.0 minutes. *11*, Aluminum wire, no current, time 4.5 minutes. *12*, Copper wire, no current, time 6 minutes.

at the neck of the original cannula), the current flowing was 1 milliampere under a pressure of 10 volts. At other times the rheostat supplied 70 to 80 volts, and the current flowing was 2 milliamperes under 20 volts pressure. The figures immediately following show the effect of the passage of 1 milliampere of current when copper wire was used as the positive pole.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Copper	44	No current	9.6	16.5	5.0
Copper	28	1 milliampere	2.9	5.5	1.0

In no experiment was the coagulation time, with the current passing, as long as the coagulation time without current.

The average coagulation time with a current of 1 milliampere passing through copper wire was reduced from the average time of copper without current by 70 per cent (see fig. 1).

When aluminum wire was used in place of copper wire, the coagulation times were shorter, but the percentage reduction was about the same.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Aluminum	32	None	5.3	9.5	2.0
Aluminum	30	1 milliampere	1.4	3.0	0.5

The average coagulation time with a current of 1 milliampere passing through aluminum was reduced from the average time of aluminum without current by 74 per cent.

When 2 milliamperes of current passed instead of one, the coagulation time was reduced still more. With copper the figures were as follows:

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Copper	44	None	9.6	16.5	5.0
Copper	10	2 milliamperes	1.9	3.0	0.5

The average clotting time with a current of 2 milliamperes passing through copper was reduced from the average time of copper without current by 80 per cent.

If aluminum replaced the copper, and the same amount of current was used—2 milliamperes—the coagulation time was shorter, and the percentage reduction almost equalled that with copper.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Aluminum	32	None	5.3	9.5	2.0
Aluminum	12	2 milliamperes	1.0	1.5	0.5

The average coagulation time with 2 milliamperes passing through aluminum was reduced from the average time of aluminum without current by 81 per cent. With the present arrangement of the apparatus, owing to the resistance imposed largely by the constriction at the neck of the original cannula, it was impossible to use greater amounts of current than 2 milliamperes. The use of 1 milliampere reduced the average clotting time with copper 70 per cent, with aluminum 74 per cent; the use of 2 milliamperes reduced the average clotting time with copper 80 per cent, with aluminum 81 per cent.

It is of interest to study the effect of the use of two factors causing reduction in clotting time—aluminum and current, as compared with copper without the passage of current.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME
			minutes
Copper	44	None	9.6
Aluminum	12	2 milliamperes	1.0

The percentage reduction of the average clotting time under these circumstances—the use of aluminum and 2 milliamperes—from the average time of copper without current was 90 per cent.

The effect of the current, 1 milliampere, passing through the soft iron wire was unexpected in view of the previous use of iron wire in electrolysis in aneurisms.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Iron	14	None	8.3	13.5	5.5
Iron	12	1 milliampere	18.7	20	9.0

In every case except two no clot was recorded at the end of twenty minutes. The observation was then discontinued, the cannula examined, and sometimes a small clot was found, sometimes not. In the two cases, the clotting times were 16 and 9 minutes. In the latter case, the two times without current were 5.5 and 7.0 minutes, and a second observation with current ran over twenty minutes.

The average coagulation time when 1 milliampere was passing through iron was increased over the average time with iron and no current by

at least 125 per cent—how much more cannot be told from the present figures.

An observation of interest in connection with the use of iron and current is the dark coloration of the blood in the cannula about the wire. This color increased and deepened as time ran on until, at the end of the twenty minutes, it was approaching black.

By using certain of the above figures, further comparison may be made between the effects of copper and aluminum.

CURRENT	COPPER	ALUMINUM	PER CENT REDUCTION
	minutes	*minutes*	
None	9.6	5.3	45
1 milliampere	2.9	1.4	52
2 milliamperes	1.9	1.0	47

The average reduction in coagulation time caused by the use of aluminum instead of copper under varied conditions of current flow was 48 per cent.

A matter of considerable importance arises in the kind of clot produced by the metals with and without electricity. In the case of iron the clots under both conditions were like those caused by the presence of a foreign body in blood. In the case of copper normal clots were found when no current was used, but with the current, the tendency was toward a small brittle charred clot, especially with the greater current. In all cases with aluminum the clot was considerably greater in amount, especially with current, and never charred. The consistency was different from that of the normal clot—was more like a gelatin, somewhat friable. The clot did not darken in color.

A single experiment was performed which is capable of quantitative development. Blood was drawn into a well vaselined vessel, so that at the end of forty-five minutes it had not clotted. Into such blood, at different times aluminum, copper and iron wires were plunged, and 2 milliamperes of current passed through them as positive poles. At the end of twenty minutes, practically no clot had formed about the iron wire; a small amount, somewhat charred, about the copper wire; and a considerable amount of gelatinous clot of good color about the aluminum wire. Such results suggest the use of a suitable aluminum wire in the treatment by galvanism of aneurisms that are anatomically favorable.

SUMMARY

When the wire used in the Cannon-Mendenhall method is aluminum instead of copper, the coagulation time of blood of Belgian hares is reduced 48 per cent.

When the wire is used as a positive pole and 1 milliampere of electricity is passing, the coagulation time is reduced 70 to 74 per cent.

When 2 milliamperes are passing, the coagulation time is reduced 80 to 81 per cent.

When the wire is aluminum instead of copper and 2 milliamperes are flowing the coagulation time is reduced 90 per cent.

When the wire is iron instead of copper or aluminum, the passage of 1 milliampere of current causes an increase in coagulation time of more than 125 per cent—how much more was not determined.

Under most conditions, but especially with current, the clot about the aluminum wire is greater in amount, and more normal in color than the clot about the copper.

My most sincere thanks are due to Dr. W. B. Cannon for his personal interest, help, and inspiration.

My thanks are due, too, to Mr. G. P. Allen who assisted me throughout these experiments with skill.

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VASOMOTOR SUMMATIONS

E. G. MARTIN AND P. G. STILES

From the Laboratory of Physiology in the Harvard Medical School

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In a previous paper (1) the authors have described experiments in which stimulation was applied to afferent paths, singly and simultaneously, to observe the effect upon the blood pressure. It was found that the stimulation of two paths at the same time may be more efficient than the employment of either of these paths alone to elicit vasomotor reactions. The summation of depressor (or excito-dilator) effects was sometimes noted, while with stronger stimuli pressor summations were often obtained. The degree of summation was moderate and it seemed more to be relied on when the nerves chosen for simultaneous stimulation were situated in remote parts of the body.

The experiments now to be reported are upon the vasomotor effects secured by stimulating two nerve-paths of which one was always the central vagus. This trunk, in the cat, usually contains the fibers which have the depressor property in the highest degree—those which are regarded as constituting *the* depressor nerve. We have published a study of the vasomotor responses obtainable from the central end of the vagus with measured intensities of stimulation (2). It has been our impression that two thresholds are crossed as the stimuli applied to the vagus are progressively increased in power: the first is the threshold of the 'mild' (excito-dilator) reaction while the second ushers in the true depressor phenomenon. By this we understand a strong inhibition of the vasoconstrictor center. The mild reaction entails a lowering of blood pressure not often exceeding 15 per cent, with a tendency to recovery during the continuance of the stimulation. The full depressor response is manifested by a fall of pressure amounting to 30 per cent or more and there is little sign of rallying even during several minutes' stimulation.

When a peripheral nerve other than the vagus is subjected to increasing stimulation we have again evidence that two thresholds are successively surmounted (Martin and Lacey, (3)). Weak stimuli produce a fall of pressure approximately similar to that secured from the vagus

while at about the same level at which the vagus begins to give the full depressor response most other nerves begin to evoke a rise of pressure. The simplest explanation has seemed to be that the lower threshold in both cases is that of the vasodilator mechanism while the higher one is that of the vasoconstrictor center. It is assumed that this center may be influenced in the direction either of inhibition or excitation and that the requisite stimuli for producing the two effects are of the same order of intensity.

Procedure. The cats were anesthetized with urethane, ether being used occasionally to deepen the narcosis. This was seldom necessary. Both vagi were cut. Stimulation was applied to the nerves by means of platinum electrodes in glass tubes, a modification of the Sherrington electrodes devised by Martin. For the simultaneous stimulation of two nerves two calibrated induction coils were used. The secondary circuits were independent while the primary coils were in series upon one circuit. This common primary circuit was interrupted by Martin's mercury key (4) actuated by a crank on a motor-driven shaft. The primary current was sometimes of 0.1 ampere, sometimes of 0.2 or 0.3. The rating of the shocks in the Z-units of Martin (5) could be found at any time by the use of calibration tables. The rate of interruption was about 8 to 12 per second. Both make and break shocks were allowed to take effect, polarization being thus minimized. The nerve most often used with the vagus was the peroneal.

Results. The summations with which we have had to deal have been sometimes algebraic and sometimes arithmetical. That is to say, we have sometimes had to do with antagonisms and sometimes with reinforcements. The reinforcements present the simpler condition and may first be discussed. At one time or another we have seen the realization of almost every theoretical possibility. For example, we have the case in which stimulation of the vagus with a certain current-strength leads to a mild fall of pressure while stimulation of the peroneal with an appropriate current causes a fall of the same order. Under such conditions we have found that the fall of pressure following simultaneous stimulation of the two nerves with the same current as before may be just about the sum of the two separate depressions.

An example of this ideal depressor summation may be given. A stimulus rated at 195Z applied to the vagus gave a drop of 8 per cent (a mild type of reaction). A stimulus rated at 406Z applied to the peroneal reduced the pressure by 12.5 per cent. When the same stimulation as before was given both to the vagus and to the peroneal at one time the resulting fall of pressure was 20.6 per cent. The combination

has a magnitude hardly ever reached with nerves other than the vagus and is in the border region between the mild and the profound reactions as exhibited with that nerve. In another case the following summation was recorded:

Stimulus to peroneal 457Z, drop	10 per cent
Stimulus to vagus 450Z, drop	22 per cent
Both together, same stimuli, drop	29 per cent

Here the united effect falls short of the sum of the two.

In a few instances the depression produced by stimulating the two nerves at once was in excess of the sum of the separate pressure reductions. For example, we have the following:

Stimulus to peroneal 406Z, drop	12.5 per cent
Stimulus to vagus 195Z, drop	9.0 per cent
Both together, same stimuli, drop	28.0 per cent

Here the vagus effect by itself is clearly of the mild type while the combined approaches the full depressor character.

A tracing here reproduced (fig. 1) shows a depressor summation on a scale of unusual magnitude. The animal was an exceptional one in that stimulation of the sciatic nerve gave pronounced lowering of the blood pressure and that no reversal of effect (pressor reaction) was obtained from this nerve with the greatest intensity of stimulation. In the record, 36 is the response to simultaneous stimulation of the vagus (1700Z) and the sciatic (2460Z). The fall is 42.5 per cent. No. 39 is a check on 36; the fall is 39 per cent. No. 37 is the response to sciatic stimulation alone (2460Z); the pressure falls 29 per cent. At 38 the vagus alone was stimulated (1700Z) and the fall was 20 per cent.

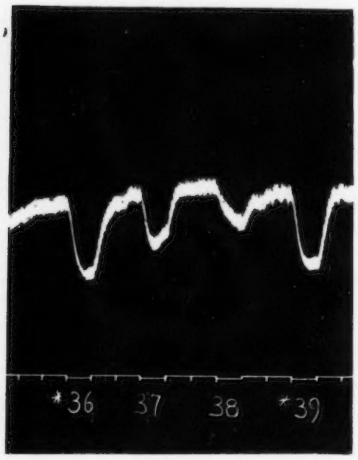

Fig. 1.

We must now consider the summation effects obtainable when the separate effect from the leg nerve is a rise of pressure. There are two distinct opportunities for investigation: the pressor reaction may be

matched against the mild lowering of pressure secured by weak excitation of the vagus or it may be attempted when the vagus stimulation is strong enough to induce, by itself, the typical depressor response. In the former combination we have found that the fall of pressure can be converted to a rise.

Bayliss (6) long ago (1893) reported the possibility of antagonizing the depressor in the rabbit by strong stimulation of another afferent nerve. His study was qualitative in character but he states that with

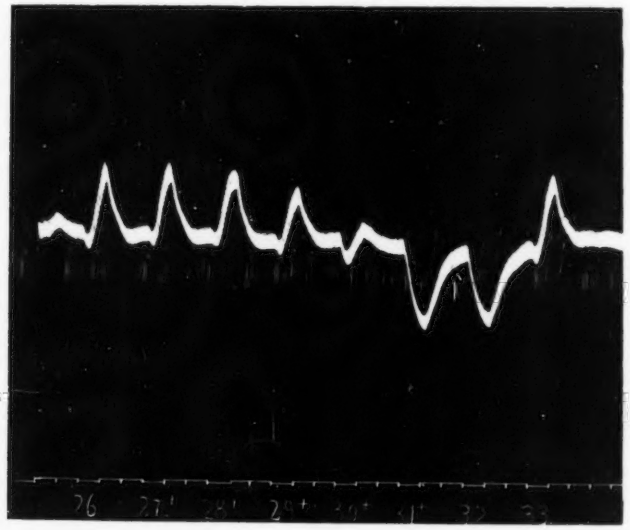

Fig. 2.

properly chosen strengths of stimulation he found it possible to obtain a neutralization of effects and to hold the blood pressure undisturbed at its original level.

We may say with equal truth that a rise of pressure can be converted to a fall. Attention may be called to the following series of results, obtained in trials in which a constant peroneal (pressor) stimulation was opposed by vagus stimulation of increasing intensity. (The tracing for the second part (nos. 26–33) is reproduced, figure 2.) The tabulation is below.

Peroneal, 990Z, vagus, 223Z, pressure rises..... 34.5 per cent
Peroneal, 990Z, vagus, 265Z, pressure rises..... 32.C per cent
Peroneal, 990Z, vagus, 400Z, pressure rises..... 14.8 per cent
Peroneal, 990Z, vagus, 554Z, pressure *falls*..... 21.0 per cent

Again:

(26) Peroneal, 670Z, pressure rises..... 24.0 per cent
(27) Peroneal, 670Z, vagus, 126Z, pressure rises..... 25.0 per cent
(28) Peroneal, 670Z, vagus, 180Z, pressure rises..... 24.0 per cent
(29) Peroneal, 670Z, vagus, 223Z, pressure rises..... 19.5 per cent
(30) Peroneal, 670Z, vagus, 265Z, pressure *falls*..... 7.0 per cent
(31) Peroneal, 670Z, vagus, 400Z, pressure *falls*..... 32.5 per cent
(32) Vagus alone, 400Z, pressure *falls*..... 29.6 per cent
(33) Peroneal alone, 670Z, pressure *rises*..... 30.0 per cent

Here we see the vagus asserting its full power to depress the blood pressure in spite of a simultaneous application of a pressor stimulus which by itself proved highly effective at the close of the experiment.

We have observed repeatedly that if we calculate the ratio between the stimulus applied to the vagus and that applied to the leg nerve when the result is a neutralization of one reaction by the other, the value obtained is roughly constant. For instance: a stimulus to the vagus rated as 400Z offset the influence of a stimulus to the peroneal rated as 990Z. The ratio $\frac{990}{400}$ is equal to 2.48. Later in the same experiment a vagus stimulus described as 265Z counterbalanced 670Z on the peroneal. The ratio $\frac{670}{265}$ is equal to 2.53. Ratios from another experiment may be given in tabular form:

1300Z on the peroneal balances 1100Z on the vagus..... Ratio, 1.18
940Z on the peroneal balances 960Z on the vagus..... Ratio, 0.98
750Z on the peroneal balances 770Z on the vagus..... Ratio, 0.98
554Z on the peroneal balances 558Z on the vagus..... Ratio, 0.99

The fact that these ratios are close to unity has no significance. We have not taken into account the unequal resistances of the two nerves. For the same reason we cannot institute comparisons between data secured in different experiments. Neither is it strange that in any one experiment the constancy of the ratio is eventually lost, for a decline in the condition of the two nerves and their connections is to be expected. The one which is first to show a rising threshold will naturally cease to interact with the other upon the original footing. The signifi-

cant thing is that even under temporary and favoring conditions the ratio should be demonstrable at all.

It remains for us to speak of the results observed when we have attempted to convert a full depressor reaction into a rise of pressure. When we apply to the vagus a stimulus strong enough to give a maximal lowering at both the beginning and the conclusion of a series of trials we find that we can lessen the vagus effect and at last make it insignificant by the simultaneous application of powerful pressor stimulation. The interesting fact is that we cannot transform it into a rise. Consider the following figures:

Vagus by itself, 558Z, pressure falls..... 45 per cent
Peroneal, 125Z, and vagus, 558Z, pressure falls..... 43 per cent
Peroneal, 265Z, and vagus, 558Z, pressure falls..... 41 per cent
Peroneal, 554Z, and vagus, 558Z, pressure falls..... 35 per cent
Peroneal, 940Z, and vagus, 558Z, pressure falls..... 30 per cent
(There was a recovery within the period of stimulation until the final level was only 12.8 per cent below the initial.)
Peroneal, 1300Z, and vagus, 558Z, pressure falls..... 16.5 per cent
(There was a recovery of the initial level but no rise above it within the period of stimulation.)
Peroneal, 1600Z, and vagus, 558Z, pressure falls..... 8 per cent
(Again there was recovery of the initial level but no rise above it.)
Peroneal, 1800Z, and vagus, 558Z, pressure practically unchanged
Peroneal, 1800Z, without the vagus, pressure *rises*..... 24 per cent

Figure 3 shows the type of record just described. At 38 the vagus alone is stimulated (430Z). The fall is 36 per cent. In each of the following trials, through 45, the same strength of stimulation is applied to the vagus. Simultaneous stimulation of the peroneal is as follows: No. 39,—125Z; No. 40,—265Z; No. 41,—554Z; No. 42,—940Z; No. 43,—1300Z; No. 44,—1600Z; No. 45,—1800Z. The depression produced by the vagus is annulled by these strong peroneal stimulations but there is no appearance of an actual elevation. No. 46 is the pressor response to peroneal stimulation (1300Z); the rise is 20 per cent. (It is to be noted that the pressure falls *after* each of the combined stimulations, 41–45. The attention for the present is to be fixed on the period of stimulation and not on the after effect.)

It is manifest from this and other cases that the strong stimulation of the leg nerve simultaneously with the vagus limits the duration of the depressor effect. It may entirely counteract that effect also but we have not seen the pressor dominant over the depressor in any instance which could be regarded as trustworthy. In rare cases where

such a reversal has seemed to appear a diminishing irritability of the vagus or its central connections was found to have been present.

After effects of stimulation. Stimulation was usually applied for periods of thirty seconds. In most cases the maximum change of blood pressure which can be looked for is recorded within this interval. But attention is to be called to an interesting exception. This is noted when strong stimulation of the vagus is balanced by strong stimulation of another nerve. The reaction during the period of excitation may be a small fall of pressure, as already indicated or in some cases a preliminary larger fall, followed within the period of stimulation by re-

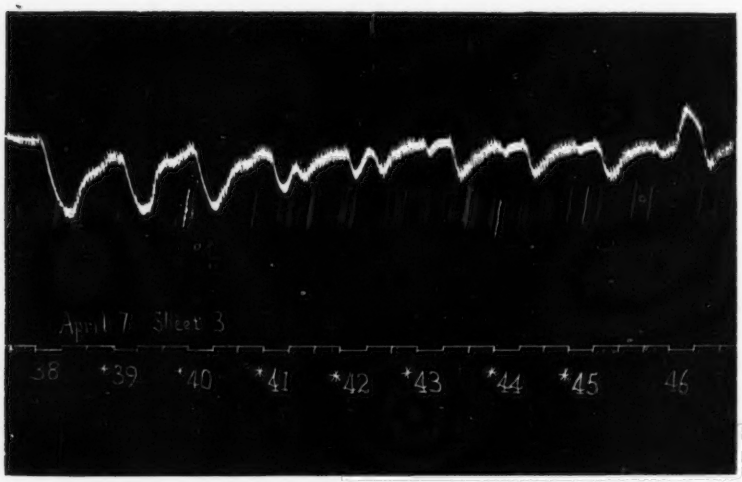

Fig. 3.

covery. When stimulation is discontinued in such instances it is commonly observed that the pressure drops considerably more than it had previously. Within another thirty seconds it returns to an average level. In other cases where the effect during stimulation is a small or moderate rise a reversal may follow, the pressure dropping far below the average value.

DISCUSSION

Depressor Summation. We have described above, experiments in which we applied to the central vagus and another afferent nerve, simultaneous stimulations of such intensity as in either case alone to

give mild (excito-dilator) depressions. In comparison with similar experiments in which two afferent nerves, exclusive of the vagus, were employed, the striking difference was the much greater degree of depressor summation obtainable with the inclusion of the vagus. Evidently the vagus is not precisely equivalent to other sensory nerves; or, to put the point in another way, the mild depression observed when the vagus is moderately stimulated does not correspond in all respects with the mild depressions obtained under similar conditions from excitation of other nerves. The difference that at once suggests itself is that in the background of the ordinary sensory nerve are fibers whose excitation will antagonize the depressor response, whereas the corresponding fibers of the vagus instead of antagonizing will accentuate it. The fact that summation is possible shows that the two nerves together act more potently on the central mechanism than either alone. Where both contain pressor fibers, it is conceivable that, although these fibers are not clearly competent, their combined action may nevertheless limit depressor summation to the moderate amount previously reported by us.

We have thought of the pressor mechanism as having a much higher threshold than the depressor; we need to bear in mind, however, that the threshold of reversal from depresssor to pressor reaction is probably higher than the actual threshold of the pressor mechanism, for before a depressor reaction can be changed to a pressor in the presence of continuous depressor excitation a degree of activity considerably above the threshold must have been attained. There would seem to be no reason why with stimuli approaching the reversal point we might not have enough pressor activity to serve as a basis for summation, even though not enough to counteract the depressor reaction. With the vagus forming one of the nerves used in evoking summed responses the depressor responses are summated but there is no corresponding summation of pressor influences. Rather, any that may be present in the ordinary nerve excited are neutralized by the vagus. Full scope is therefore given for the development of marked depressor reactions. The case cited by us in figure 1, where the pressor influence of the sciatic nerve was apparently in abeyance, illustrates the same point more emphatically. In this instance, because there was no pressor tendency, much stronger stimuli could be applied than was usually possible in these experiments (1). The depressor summations obtained were correspondingly more pronounced.

Summation of antagonistic influences. When afferent nerves other

than the vagus are strongly excited pressor reactions are, as a rule, to be obtained. We have shown that these can be successfully antagonized by suitable vagus excitations. Scrutiny of the conditions of antagonism brings out strongly the remarkable constancy of operation of what is thought of as an exceedingly complex mechanism. W. T. Porter (1910) (7) has previously emphasized this constancy of vasomotor responses. Under ordinary experimental conditions the mechanism must be pictured as in an equilibrium which includes a fairly steady vigor of discharge of vasomotor impulses; these establishing the condition that we describe as vasomotor tone. Afferent stimulation disturbs this equilibrium; either upward or downward according to the nature of the stimuli. Our experiments show that a disturbance upward can be neutralized by a suitable depressor influence, and furthermore, that the strength of depressor stimulation necessary for its neutralization bears, in any experiment, a fixed ratio to the strength of the excitation by which the elevation of tone was brought about. In this feature we emphasize, even more strikingly than does Sherrington (8) in his experiments on antagonism of skeletal muscle reflexes, the "algebraic summation" which characterizes the reaction of the central nervous system to opposing stimulations.

A feature of the antagonism which is possibly significant is that in general the depressor influence is more potent than the pressor. This statement may appear inconsistent with our previous emphasis on the mathematical relationship existing between the two influences. What we desire to point out, however, is this: although the amount of depressor stimulation necessary to neutralize any given pressor influence is in fixed proportion to the pressor influence, if a stronger depressor stimulus be applied than that which just balances the pressor the resulting depression is disproportionately great. This we have seen repeatedly. An instance appears in figure 2, where a pressor stimulus of 670Z which by itself caused a substantial rise of pressure (nos. 26 and 33) and required for neutralization a vagus excitation of 265Z (no. 30) was apparently wholly ineffective when the vagus stimulus was increased to 400Z (no. 31). A parallel case in which the antagonistic factor was asphyxia has been reported by Asher (1906) (9).

Another line of evidence which suggests the prepotency of the depressor is exemplified in figure 3, in which a very considerable increase in pressor stimulation strength beyond that required to neutralize a strong depressor effect did not carry the blood pressure above the level of neutralization. This latter observation, in fact, shows clearly that

the blood pressure level is not determined altogether by the algebraic sum of all the afferent impulses calculated to modify it. The vigor of discharge which determines vasomotor tone may be restored by strong pressor stimulation when strong depressor excitation is tending to lower it, but in the presence of such depressor excitation it is difficult if not impossible to force a vigor of discharge greater than that of normal vasomotor tone. If an illustration may be ventured, the vasomotor tone may be compared with the level of water in a tank which has a supply-pipe and an overflow. Increasing the supply (pressor stimulation) may force the level above that of the waste-way. Depressor stimulation is analogous to draining the water by opening an outlet at a lower level than that of the ordinary overflow. When this extraordinary draining of the water is in progress it is easy to imagine that driving the feed-pump to its limit may restore the normal height of the surface but cannot raise it higher because of the double escape which becomes available (fig. 4). The final explanation of this phenomenon must wait for knowledge beyond that which we now possess of the detailed functioning of the central nervous system.

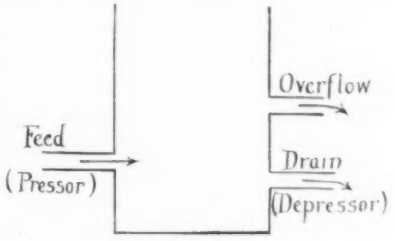

Fig. 4.

The depressor after effect. Our observation that the after effect of combined strong pressor and depressor stimulation is a manifestation of persistent depressor influence appears at first view to be at variance with the observations of Baxt (1875) (10) on the heart and of v. Frey (1876) (11) on the submaxillary gland, in which opposing peripheral influences were excited simultaneously, and in which the *stimulation* phase was the one to appear after cessation of excitation. Asher, in the paper cited above (9), reports that the asphyxial pressor influence is the one to show itself when asphyxia and depressor excitation are withdrawn simultaneously. In the single protocol given by Asher (loc. cit., p. 94) the asphyxial rise is recorded as reaching its height two seconds after the release of the tracheal clamp, an interval scarcely long enough to have permitted adequate oxygenation of the vasomotor center. It is possible, therefore, that there was not in that case a true after effect, but an actual persistence of asphyxial excitation, after the withdrawal of the depressor stimulation. A well-known feature of the

response of the vasomotor mechanism to strong depressor stimulation is the sluggishness of the return to the former level. This is well illustrated in figure 3, no. 38. Such a curve as no. 43 in the same figure suggests that the depressor influence although not able to bring about the usual change in blood pressure in the presence of strong pressor excitation, is nevertheless acting on the mechanism in precisely its usual fashion. Immediately upon the withdrawal of the pressor influence the blood pressure takes the position it would have had at the same moment had there been no pressor factor. The return to the normal level is of the same degree of sluggishness as after depressor stimulation alone. This view of the nature of the inhibition of the vasomotor center by depressor excitation places it in Heidenhain's (12) second class of inhibitory actions, the class in which inhibition and augmentation act on different parts of the affected mechanism. Asher (loc. cit., p. 96) takes this same position.

On the other hand, our observations discussed above, on the "algebraic summation" of pressor and depressor influences, would appear to place the vasomotor center in harmony with the center for skeletal muscle reflexes, in which, as stated, Sherrington has shown the principle of algebraic summation to obtain, and in which on that account, he postulates the inhibition to be of Heidenhain's *first* class, a direct opposition to excitatory influences. If our interpretation of the inhibitory after effect is sound, we shall be led to the conclusion that exciting and inhibitory influences may act simultaneously upon a mechanism in such a manner that each exerts its normal effect in the presence of, but masked by, the other, and yet be so related as to show very exact algebraic summation.

SUMMARY

1. Depressor (excito-dilator) summation is more pronounced when one of the afferent nerves excited is the vago-depressor trunk than when the summation is secured by stimulation of two afferent nerves other than the vagus.

The suggestion is offered that the presence in afferent nerve-trunks generally of pressor fibers may limit the degree of excito-dilator summation obtainable through excitation of such trunks. The corresponding fibers in the vagus are depressor, and tend to favor, rather than to limit excito-dilator summation.

2. The antagonism between pressor and depressor influences is shown to have the character of algebraic summation when the intensity of

depressor stimulations necessary to neutralize a series of pressor influences is taken as the criterion.

3. Depressor stimuli stronger than just sufficient to overcome concurrent pressor influences have an effect disproportionate to their mathematical superiority.

4. Strong depressor stimuli can be neutralized by strong pressor excitation, but it is difficult, if not impossible, to force blood pressure above the normal level thereby.

5. The after effect of pressor-depressor summation is typically a transient fall of blood pressure. The appearance of the curve suggests that the depressor influence is masked but not destroyed by concurrent pressor excitation.

6. The nature of the after effect indicates that depressor and pressor influences act upon different parts of the central mechanism. The algebraic summation has been formerly interpreted to mean opposing action at a common point. The suggestion is made that action at different points may be consistent with algebraic summations.

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THE MOVEMENTS OF THE MITRAL CUSPS IN RELATION TO THE CARDIAC CYCLE

ARCHIE L. DEAN, JR.

From the Physiological Laboratory, Cornell University Medical College, New York City

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I. INTRODUCTION

The generally accepted conception regarding the movements of the auricular-ventricular valves in the beating heart, as stated in several modern text books of physiology, may be summarized as follows: When the auricle contracts it forces a small quantity of blood into the relaxed ventricle. This raises the intra-ventricular pressure a trifle, and by forming eddies behind the valve-cusps causes their approximation or partial closure. Thereupon follows ventricular systole with the immediate rise of intra-ventricular pressure. When the intra-ventricular exceeds but slightly the intra-auricular pressure, complete closure of the auriculo-ventricular valves occurs, and regurgitation is prevented.

The accuracy of this conception has recently been reinvestigated by Henderson and Johnson (1) who mounted in a glass jar the excised valves of ox hearts, in such a way that the natural connections of the valves were undisturbed. The action of these valves could be clearly observed when pressure changes, simulating those within the heart, were artificially produced from either the auricular or the ventricular side. The chief conclusions drawn from this study were:

1. Auricular systole forces a jet of blood through the auriculo-ventricular openings. When this jet breaks at the end of auricular systole, a zone of negative pressure is formed between the cusps. This unrolls the edges of the valves, approximating them in complete and final closure just previous to ventricular systole. It is this method of closure which, according to these investigators, prevents regurgitation in the normally beating heart.

2. When ventricular systole is not preceded by an auricular contraction in the cycle (as, for example, in auricular fibrillation), the rise of intra-ventricular pressure closes the valves. This type of closure is, however, abnormal in that the valves do not unroll, but close by a "hinge movement" which is necessarily accompanied by a slight regurgitation.

If these conceptions of Henderson and Johnson be correct, we must revise our current ideas not only of the mechanism of valve movement, but also of the temporal relation of valve closure to auricular and ventricular systoles. For example, it is necessary to assume that the valves close at the end of auricular systole and before ventricular contraction begins. If this be the case, it may offer an explanation as Lewis has suggested, of the pre-systolic sound heard whenever the a-v interval is long (2). Furthermore, if their view is correct that ventricular systole itself is not directly responsible for the valve closure, we may be compelled to modify our views as to the dual origin of the first heart sound.

From a physiological as well as a clinical standpoint, therefore, it is desirable to again investigate the question as to the movements of the valves within the beating heart, and the precise relation of their movements to the cardiac cycle.

II. METHOD

Doubtless the most accurate and reliable method of studying this question would be to attach directly to the valves of the intact heart freely movable threads or hairs, and transmit their vibrations directly to recording levers capable of accurately following the slightest valve oscillation. This, so far, has not proven feasible. After considerable preliminary experimentation, however, it was found possible to study the valve action of the perfused cat's heart in this way. The description of the method naturally divides itself into a consideration of (1) the preparation of the heart and (2) the method of recording.

Preparation of the heart. The chest of an etherized cat is opened and the animal allowed to die by asphyxia. The cat heart is chosen because of anatomical advantages, and this method of causing death is used because comparatively little harm is done the heart. When the heart has ceased beating, it is removed from the chest, care being exercised to leave enough of the venae cavae and aorta attached. As soon as possible, a T-cannula (*P*) is fastened into the aorta (*O*),

and warmed Locke's solution is perfused through the heart under a suitably gauged pressure (fig. 1). A T-cannula is used so that one limb, not tied into the aorta, transmits Locke's solution from the main resevoir, the other, fitted with a thin-walled, partially clamped, rubber tube, serves as a continuation of the elastic aorta (*Q*). Thus the ventricle contracts against an aortic pressure which varies during each cycle as in the intact heart.

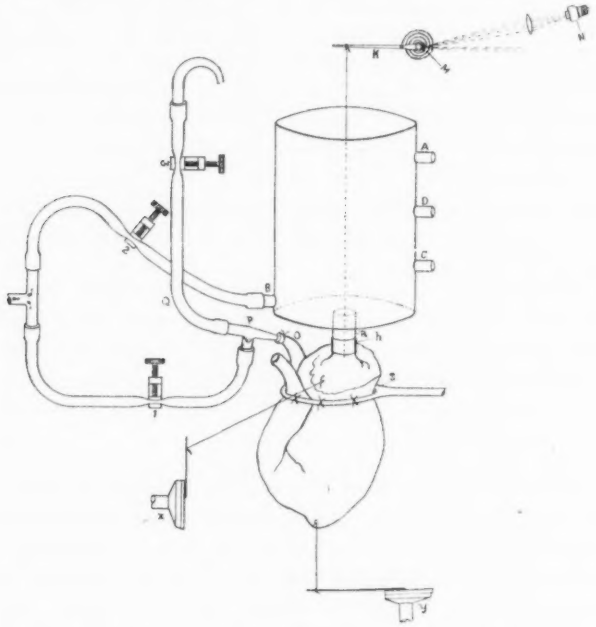

Fig. 1. Diagram of apparatus—description in text.

Next, an opening about 3 mm. in diameter is cut just posterior to the left auricular appendage, and the pulmonary veins ligated. With a small, curved needle, possessing an eye near its point, a human hair is passed downward, then upward through the medial or septal cusp of the mitral valve near its free margin, and there securely tied. Only a very small portion of the cusp is caught in the ligature. After many trials it was possible to catch the same point of the cusp in each experiment. This was verified by subsequent examination. The short

end of the hair is next cut off close to the cusp to avoid extraneous contacts.

As a means of maintaining an auricular pressure which, at the same time, could be modified at will, the glass reservoir-cylinder shown in figure 1 was devised. Around the bottom exit (*R*), adhesive plaster is wrapped. Between this and the opening made into the left auricle, an impervious suture is made, the hair attached to the valve having been previously led upward through the opening. Through a low lateral opening (*B*) of the reservoir, perfusing fluid enters, and by stopping either of the lateral exits (*C*, *D*, or *A*), the auricular pressure may be varied in steps of 20 mm. of Locke's solution. In this way pressures varying between 45 and 85 mm. of fluid were maintained.

Ligatures are placed at suitable intervals through the musculature of the heart at the level of the auriculo-ventricular ring. By these the heart is anchored to a rigid and neatly fitted ring of metal (*S*) through which it is suspended. Thus the auriculo-ventricular ring does not change its position while the heart beats. This procedure is necessary in order to prevent movements of the cardiac musculature from being registered through the thread attached to the cusp. Care must be taken, of course, not to exert such tension upon these threads as to cause any relative valvular insufficiency, nor may any but very small blood vessels be caught within the ligatures.

Method of recording. The contractions of the left auricle and left ventricle are recorded optically on a moving bromide film, by connecting them through threads to the straw levers of recording segment tambours (*x-y*). These communicate by rubber tubes with Frank's recording segment capsules (*N*).[1]

The movements of the septal cusp of the mitral valve are communicated by the hair to a light lever (*K*), rotating about a fixed axis and held up by a tiny coil very much as in Frank's recording manometer. The rotation of this axis is recorded by reflecting a beam of light into the tiny mirror (*M*) fastened directly to this axis.

The common source of light for this mirror as well as those of the segment capsules was Frank's arrangement of a Nernst filament, whereby three aligned beams of light are projected, and each focused upon its mirror. The mirrors in their turn reflect beams of light through the slit of a photokymograph and so record upon moving bromide paper.

[1] For a description of these capsules see Wiggers: Journ. Amer. Med. Assn., 1915, lxiv, 1485.

III. VALVE MOVEMENTS WHEN AURICLES OR VENTRICLES BEAT ALONE

To facilitate the analysis of records of normally beating hearts, it seems desirable to first study the movements of the valves when the auricles and ventricles beat separately. The cases so analysed represent in part those instances in the perfusion experiments in which the auricles or ventricles beat alone. Such occasions are always found in any series of perfusion experiments, but here only records were considered in which there was active beating of the auricles without any signs of ventricular movement, or vice versa. Furthermore, it was easily possible at the end of the experiments, to fibrillate the auricles or ventricles with a tetanizing electric current and in this way study their separate effects.

The movements of the valves *when the auricles beat alone* are illustrated in the two segments of figure 2. Two types of movement were recorded. As shown in curve A a short interval after auricular systole begins, but before its termination, an upward oscillation of the cusps occurs (cf. points *1* and *2*). This is followed during relaxation of the auricle by a downward movement *3–4* and an after-vibration of the valves (*4*, *5*, and *6*). The amplitude of the initial and after-vibrations is somewhat dependent upon the intra-auricular pressure. The after-oscillation is not at all typical of the cusp movement in the majority of experiments, and, apparently, only occurred when the ventricles were extremely flaccid. The usual form of movement is a single vibration, during auricular contraction and relaxation, as shown in figure 2*B*. This is preceded in most instances by a very slight downward movement (*1*, *2*) synchronous with the beginning of auricular systole. It is difficult to say whether the sharp, superimposed vibration of shorter period (*X*), only occasionally present, is due to valve vibration or is of instrumental origin since its period corresponds suspiciously well with that of the lever system used.

In none of the curves was there found any evidence that the movement toward closure, caused by auricular activity, was anything but a transient oscillation, absolutely incapable of being the efficient valve closure mechanism, not only because the valves were not maintained in a closed state, but also because effective apposition of the cusps did not occur. This could be observed ocularly.

When the ventricles beat alone the curve of valve closure is entirely different as is shown in figure 3. At the onset of systole (*1*) the valves move upward quickly, and are completely closed an instant before the

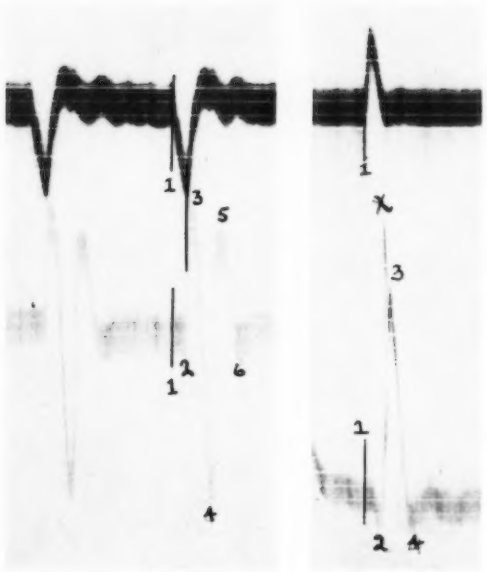

Fig. 2. Two segments of records showing oscillations of mitral cusps when auricles beat alone. *A*, Upper curve, auricular myogram (systole downstroke)—lower curve valve movements. Intraauricular pressure, 60 mm. Locke's solution. Position of points indicated by line 1. *B*, Upper curve, auricular myogram (systole upstroke)—lower curve, valve movements. Rise of curve indicates movement of valves aurieleward. Intraauricular pressure, 75 mm. Locke's solution.

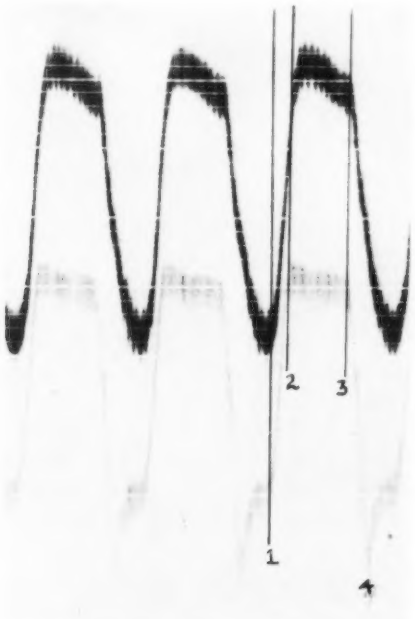

Fig. 3. Upper curve ventricular systole (rise) begins at *1* and terminates at *3*. Lower curve, movements of mitral cusp, upstroke signifies aurieleward movement.

maximum of ventricular contraction as recorded from the apex (*2*). The valves remain closed throughout systole, giving the curve a plateau form (*2–3*). Synchronous with relaxation (*3*) the valves open quickly, moving down to a lower position than they occupied before the onset of systole (*4*). From this point they are slowly buoyed upward as blood flows into the ventricle from the auricle during the remainder of diastole. Vibrations such as might account for the first sound were never found to be superimposed on the curve of valve closure when the ventricle beat without the auricle.

IV. VALVE MOVEMENTS DURING THE NORMAL SEQUENCE

When auricles and ventricles beat in normal sequence the curves show essentially the combined types of movement described separately above. Figure 4 shows the curve from an experiment where a great magnification of valve movement occurred. Auricular systole begins at point *A*, and is accompanied by a slight downward movement of the valve-cusp record. Just before the end of auricular systole the mitral valve rises sharply (*B*) and returns promptly at the onset of auricular diastole (*C*). The valve does not fall back as far as before, probably because of increased intra-ventricular pressure. Now comes ventricular systole (*D*) when the mitral cusps quickly rise to effective closure and remain in this condition until ventricular relaxation begins, when they rapidly open. A rebound often occurs as is shown in figure 4 by oscillation (*E*). No other diastolic changes occur when, as in this instance, the heart rate is rapid and the period of diastasis therefore practically absent.

Figure 5 illustrates even better the nature of the cusp movements since less magnification was used. In this case the heart rate was also slower, and a period of diastasis (*G–A*) occurred. The valve movements alone are here reproduced. If it is borne in mind that every upstroke of the curve indicates an upward, and every down stroke a downward movement of the valve cusps, we can readily obtain a vivid mental picture of the exact movements undergone by the mitral cusps in the normal cycle. It is clear that auricular systole and ventricular systole each produce in successive order an upward or closure movement of the valves. With the onset of auricular systole, probably due to the active impingement of blood upon the mitral flaps, a slight downward movement (*AB*) usually takes place. Then, near the end of auricular systole, the valves move upward toward the position of clos-

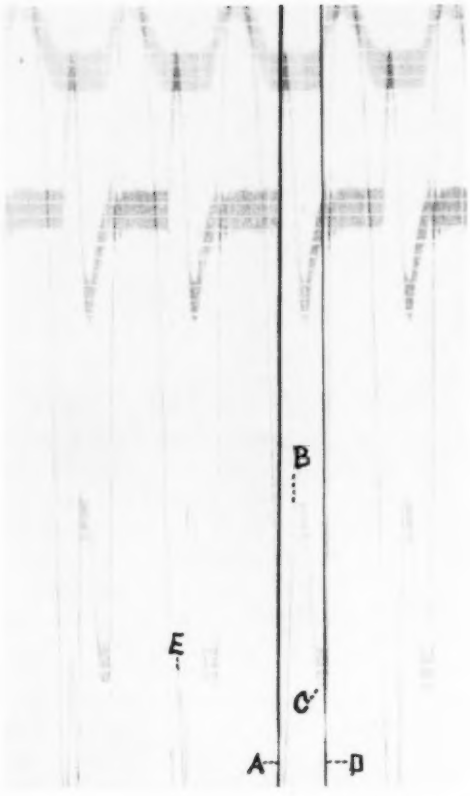

Fig. 4. Uppermost curve, ventricular systole (upstroke), middle curve, auricular systole (upstroke)—lower record, movements of mitral cusps. Speed of paper 28 mm. per second.

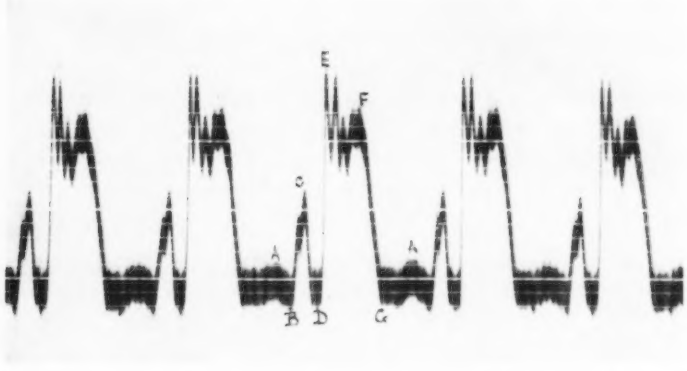

Fig. 5. Curve showing movements of mitral valves when normal sequence and a long As-Vs interval obtains. *AC*, auricular closure of valves, *DE*, ventricular closure.

ure (*C*). This closure is, however, only temporary and not complete, the valves returning promptly to their former open position (*D*). At the onset of ventricular systole, the valves are closed completely (*E*), and remain so during systole (*EF*).[2] Having returned to a point in early diastole lower than they occupied previous to the onset of ventricular systole (*G*), the cusps gradually rise auricleward during the period of diastasis (*GA*).

V. RELATION OF VALVE MOVEMENTS TO LENGTH OF As-Vs INTERVAL

In the curves shown in figures 4 and 5 the As-Vs interval often measured 0.288 seconds, a period considerably longer than is assumed to exist in the human heart from electrocardiographic studies (0.13–0.18 sec.). This long interval made these records especially valuable for analysing the several factors involved in valve closure. The question arises, however, whether two closure movements of the valves can occur in every cardiac cycle when the As-Vs interval is shorter. It is clear as shown diagramatically in figure 6, that when the As-Vs interval is progressively shortened, the curve representing the ventricular closure of the valves first follows the auricular at a shorter interval (fig. 6 *B*), then becomes supported on the auricular curve (fig. 6 *C*), and finally becomes continuous with it (fig. 6 D). For descriptive purposes these forms of closure may be designated as types *A*, *B*, *C*, and *D*.

In interpreting the valve movements of the intact heart, therefore, it is important to determine (1) how short the As-Vs interval must become before the ventricular closure begins to be supported on the auricular, (type *C*), and (2) how short the interval must become before the influences of auricular and ventricular systole blend in a single closure movement (type D).

Computations from a number of different experiments show that auricular systole precedes the auricular cusp vibration by about 0.084 seconds, and that the auricular closure of the valves, itself requires on an average 0.063 seconds (fig. 4, *AB*). It is apparent from these results that unless the As-Vs interval is greater than the sum of these figures (0.084 seconds + 0.063 seconds, i.e., 0.147 seconds), the valves have not time to reopen before ventricular systole, and the closure movement

[2] It is not possible at present to interpret the significance of the smaller oscillations superimposed upon the main curve—whether they are vibrations of the closed valves, responsible in part for the first heart sound will be investigated in a future research.

takes the form of type D (fig. 6). This closure is initiated by auricular systole, but is completed and maintained by ventricular contraction. As soon as the As-Vs interval becomes only a trifle greater, however, the valves, after auricular systole, tend to open to a slight degree, and, as this interval becomes progressively longer, the valves open more and more before ventricular closure (type C). Since auricular systole precedes the onset of auricular closure by 0.084 seconds, and the closure plus opening requires 0.188 seconds, it is evident that complete opening does not occur unless the entire As-Vs interval equals at least the sum of these figures or 0.272 seconds.

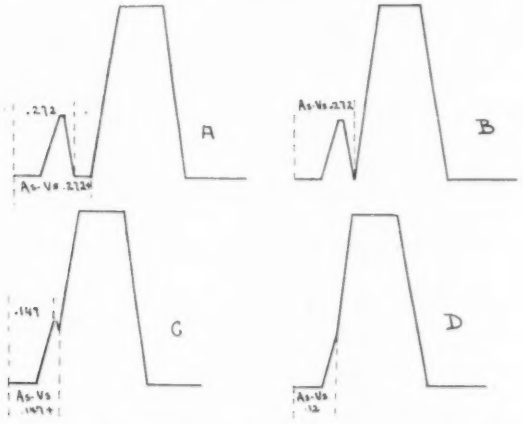

Fig. 6. Schematic drawing showing influence of the length of the As-Vs interval on the valve movements. Types *A* and *B* occur when the As-Vs interval exceeds 0.272 seconds. Type C occurs when the interval ranges between 0.147 and 0.272 seconds. Type *D* occurs when the interval is less than 0.147 seconds.

VI. SUMMARY OF RESULTS

1. By making a direct connection between the septal cusp of the mitral valve and a lever delicate enough to register its slightest oscillations, it was possible to record optically the movements of the mitral cusps as they occurred in the cycle of a perfused cat's heart.

2. When the As-Vs interval averaged 0.272 seconds or more (fig. 6, *A*, *B*), the movements of the mitral cusps during each phase of the normal cycle were as follows:

a. A short period after the onset of auricular systole the cusps move ventricleward slightly. Toward the end of auricular systole they move auricleward quickly and markedly, but not to a position of complete closure.

b. At the onset of auricular diastole the cusps quickly move ventricleward, the rapidity depending upon the existing intra auricular pressure. When ventricular tonus is low, intra-ventricular pressure is low, and when this obtains there is a rebound of the cusps from the ventricular walls. The valves remain open until ventricular systole begins.

c. At the onset of ventricular systole the cusps immediately move upward to a condition of complete closure, and remain so until ventricular relaxation begins.

d. During active relaxation of the ventricles the cusps move downward to a lower position than they occupied at the beginning of systole. From this position they gradually float upward during diastasis.

3. The sequence of movements above described also occurs when the As-Vs interval ranges from 0.147 to 0.272 seconds, except that time is lacking for a complete opening of the valves before ventricular systole again causes their closure (fig. 6 *C*). The valves open slightly during the intersystolic period, the extent increasing with the As-Vs interval.

4. When the As-Vs interval is less than 0.147 seconds (fig. 6 *D*), the valves are in the process of closing due to the auricular effect when ventricular systole begins. Hence this cardiac event merely completes the closure already initiated by the auricle. There is in this case only a single closure movement, beginning before ventricular systole—a single movement, due in part to auricular contraction and in part to ventricular contraction.

VII. PHYSIOLOGICAL APPLICATION OF RESULTS

Since any conception as to the mechanisms producing valve closure must be correllated with the temporal relations of their closure to the events in the cardiac cycle, it is appropriate to discuss briefly the bearing of these results upon different theories of valve closure.

It has been shown that the mitral cusps first begin to close after auricular systole is well under way. Several possible mechanisms may account for the closure at this time. In the first place, it may be attributed to the sudden breaking of the jet of blood injected by the auricle into the ventricle, not in the manner suggested by Henderson

and Johnson to effect the final and complete closure but rather that it tends to float the valves temporarily into position. Secondly, it is possible that the auricular contraction wave at this time reaches the muscle fibers within the valve cusps, and aids in their closure. The observation of Erlanger (4) that these muscle fibers are capable of contracting is the basis of this possibility. Thirdly, it is possible that the fall of intra-auricular pressure shown by Wiggers to occur in the middle of auricular systole (3) causes the cusps to move auricleward.

It has been demonstrated that the auricular closure of the mitral valves is incomplete and temporary—that a ventricular systole is required to effect complete closure and maintain the valve in this position until the onset of ventricular diastole. The facts that the valves close at the onset of ventricular systole irrespective of whether an auricular systole preceded, and open precisely at the beginning of ventricular diastole, lends support to the commonly accepted idea that the difference of pressure on the two sides of the valves is the chief factor in producing their movements.

It is commonly taught that the mitral cusps are approximated at the onset of ventricular systole. It has been shown in this work that some degree of approximation obtains when ventricular systole begins if the As-Vs interval falls within limits that may be considered normal (0.13–0.18 seconds). It has been further shown, however, that the extent of this approximation is directly related to the length of the As-Vs interval, so that when the As-Vs interval equals 0.272 (fig. 6 *B*) the cusps are as widely separated as at the onset of auricular systole. It is of practical importance to recognize that in cases of delayed A–V conduction the valves may undergo two distinct movements of closure, the first near the end of auricular systole, the second at the beginning of ventricular systole.

The writer wishes to express his great appreciation for the kind advice and criticism of Dr. Carl J. Wiggers.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

I. The Auricular Myogram and Auricular Systole

CARL J. WIGGERS

From the Physiological Laboratory, Cornell University Medical College, New York City

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INTRODUCTION

It is commonly believed that the auricular myogram is a graphic record of auricular systole and diastole. Such a tracing may be obtained from the mammalian auricle in several ways. Of these, the transmission of auricular movements to simple levers or tambour systems is probably the procedure most frequently employed. A small button is placed on the auricle and its movements communicated, sphygmograph fashion, to a recording lever (Ludwig and Hoffa (1)); or a point on the auricular surface is connected by a thread with a light lever or tambour system, i.e., the so-called "suspension system" is used (Gaskell (2), Englemann (3)).

As generally employed in mammalian experiments such procedures introduce two errors. In the first place, the vibration frequency of this ponderable system is so low that the records obtained are necessarily distorted by an interference of the instrument's own vibrations. This is, however, a matter of minor importance, since it is questionable whether the movements of a point or spot on the auricular surface, communicated to the most ideal lever system gives reliable evidence of the state of muscular activity which we seek to record. The auricle has no fixed point from which to contract, hence the movement of any point on its surface is modified by many factors (e.g., by lung inflation, auricular distension, changes in form, ventricular position changes, etc.) besides the contraction and relaxation of auricular fibers. It has not always been adequately recognized that changes in auricular form or position do not necessarily occur in unison with contraction and relaxation processes; that a record of one event may not be a criterion of the other.

Recognizing these facts, a number of investigators have sought to obviate the error by recording the approximation and recession of two selected points on the auricle, independent of its position changes. To this end, myocardiographs of different design have been introduced (Roy (4), Cushny (5), Wiggers (6), Gesell (7)). These instruments have the common fault that their vibration frequency is entirely inadequate, and most of them have the additional drawback that their great mass may react upon and interfere with a normal action of the thin-walled auricle. "Die Massen der beweglichen Teile" says Frank (8) "sind viel zu gross, als dass nicht Trägheitskräfte entstehen müssten, die zu einer, die der Herzthätigkeit vollständig verändernden Rükwirkung führen müssten."

APPARATUS—THE MINIATURE MYOCARDIOGRAPH

Inasmuch as an exact myogram of auricular contraction and relaxation was demanded in a careful study of questions pertaining to the temporal relations and dynamic importance of the auricle, a miniature myocardiograph was designed which was capable of accurately following the variations in the length of auricular fibers, independent of changes in auricular form, volume or position but was incapable of reacting upon the auricle so as to make its action unnatural. A high vibration frequency and small mass are two qualifications of this apparatus. The instrument, shown in natural size in figure 1, weighs less than 2 grams and is supported by a very light spring, enabling it to follow varying degrees of auricular distension and movement without affecting the contraction curve. Connected with a Frank segment capsule, the system can have a frequency as great as 118 per second.

In the design of this myocardiograph, joints, pivots and fixed axes, which cause irregular friction and add weight, are entirely eliminated. This was made possible by using a light aluminum segment capsule, 2 cm. in diameter, covered by light but tensely stretched rubber dam. As in Frank's recording capsule, a trapezoidal aluminum plate is cemented to the rubber so that it pivots upon the chord side. This plate carries a light extension arm (1.5 cm. long) with an eyelet at its end. A second similar arm is rigidly fastened to the body of the capsule. These two arms, which may be bent so that the distance between the two eyelets varies from 3 to 25 cm. are stitched to two points on the auricular surface. The approximation of these two points causes a negative pressure in the cardiograph capsule and in the connected

Fig. 1. Photograph of miniature myocardiograph (actual size).

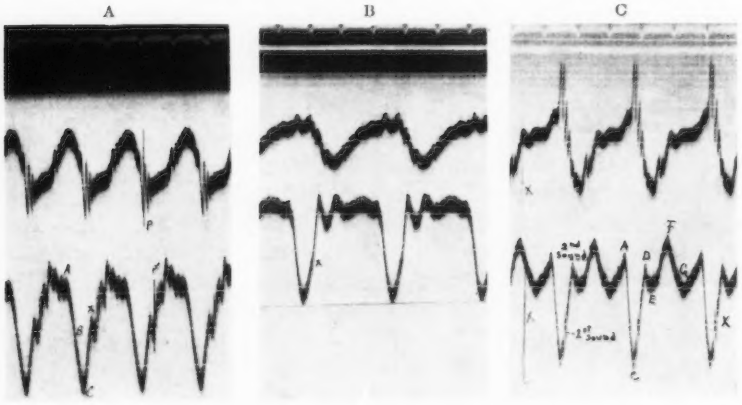

Fig. 2. *A*, *B*, *C*. Segments of myograms taken from mid-auricular region from points 5 to 7 mm. apart.

recording capsule of Frank, hence the curves move downward during contraction and upward during relaxation. In attaching the instrument, the heart is left intact within the pericardium, a window being cut over that portion of the auricle to which the apparatus is fastened.

THE CONTOUR OF THE AURICULAR MYOGRAM

The secondary or communicated oscillations in optical myograms. It was anticipated that the myogram recorded by the miniature myocardiograph would present a very simple contour. This proved to be the case only when the auricular beat was neither preceded nor followed by a ventricular systole. Such instances are shown in waves 12 to 16 of figure 3 taken during excitation of the left vagus which had produced a complete a–v block. The curves reach their maximum contraction or trough on an average, in 0.083 second (table 1). This maximum contraction is maintained just for a moment and then the curve regains its full relaxation within approximately the same time interval. No evidence of a sustained contraction or "plateau" is present.

When the auricular beat is followed by ventricular contraction (figs. 2 to 3) the contour of the relaxation curve is altered by superimposed waves. Thus, in the three segments shown in figure 2, the ascending limb contains one or two groups of such superimposed oscillations. First, in point of time, there occurs, shortly after the onset of relaxation (X) a jog, a notch, or a series of distinct vibrations. Comparisons with the ventricular cardiogram in which the vibrations of the two sounds are present, show that this corresponds exactly with the vibrations of the first sound (p–p'). When the heart is vigorous, as after partial asphyxiation (fig. 2 C), distinct sound vibrations may be transmitted to and recorded by the instrument. It may be pointed out, parenthetically, that the ability of the recording system to reproduce such vibrations is in itself a test of its adequacy.[1]

Frequently, but not constantly, a second notch occurs toward the end of relaxation (fig. 2 C, D, E). This is apparently due to a traction from the ventricle so exerted as to cause the two points to which the myocardiograph is attached to approximate. Comparison with the

[1] It is of incidental interest to note that, when an apparatus with a low inherent frequency is used these vibrations either do not occur on the record owing to a "lever throw" or with greater damping of the apparatus the movement of the lever is arrested temporarily thus giving the curve a flattened contour suggesting a plateau. This may possibly account for the plateau curves obtained by some investigators (cf. e.g., the recent results of Ewing (9)).

intraventricular pressure curves (to be published in a subsequent paper) show that this takes place during the early portion of the ejection period. Lastly, with the onset of ventricular diastole at *E* and again with the diastolic inflow from the auricle at the opening of the a–v valves (*F*), the auricular curves may be modified by ventricular action.

It is clear that the rebounds and position changes of the ventricular base consequent to its contraction and relaxation cause a series of slight expansions and contractions of the elastic auricle, which lies upon this base. These superimposed waves distort the correct myogram curves of auricular activity. They may vary in amplitude and number, depending partly on the vigor of ventricular systole, partly, however, on the proximity of the auricular myograph to the a–v junction. Since they are a part of the auricular movement they cannot be eliminated from any record taken by an efficient myocardiograph.

In themselves these oscillations are of no importance and would not merit the detailed discussion accorded them but for the fact that a clear understanding of their inherent cardiac and not instrumental origin makes certain the conclusion that when the heart chambers are beating in normal sequence, the contraction phase of the myogram curve alone can be recorded free from secondary vibrations of extra-auricular origin.

The mechanical as related to the fractionate contraction. It is quite certain that all the units of cardiac muscle lying between two points on the auricular surface neither begin nor cease to contract at the same time. On the contrary, to judge from the spread of the excitation wave, as established by Eyster and Meek (10) and Lewis, Meakins and White (11), the mechanical contraction recorded by a myocardiograph is the resultant of a more or less orderly series of contractions and relaxation which the fractional portions of cardiac tissue between two points undergo. We must, therefore, distinguish between the recorded *mechanical contraction* and the *fractionate contraction*, i.e., the interval during which any unit of cardiac syncitium remains in the contracted state.

Since the excitation wave, according to the American and English investigators above mentioned, spreads from the sinus node to the more distant portions of the auricle at the rate of approximately 1000 mm. per second, we should expect that the cardiac tissue underlying the more proximately placed arm of a myocardiograph starts its contraction before the tissue beneath the more distant arm. Within a

very short interval after the onset of the contraction, the two points on the auricular surface may be expected to approximate. As more and more fractional portions of the muscle between the two arms enter into their contraction phase, we may presume that the two points approximate with a greater velocity. Lastly, when the fractionate contractions of the more proximal portions change progressively to relaxation phases, while the more distal portions remain shortened, we may expect a progressive decrease in the rate at which the two points approximate until finally, as the fractionate contractions and relaxations are equally balanced, the peak of the mechanical curve is reached.

A careful consideration of the optical myogram taken on rapid paper affords evidence that these events affect the contour of the contraction curve. Thus, as shown in waves 5 and 6 of figure 3, the mechanical contraction (A–C) can be divided by the changes in gradient, into three distinct phases. These are:

1. A proto-systolic phase lasting about 0.02 second during which the rate of contraction gradually accelerates (AA'). As interpreted by the diagram inscribed on the curve, this phase probably represents the spread of the fractionate contractions from the proximal to the distal point.

2. A meso-systolic phase ($A'B$) lasting about 0.024 second during which contraction proceeds at a uniform though maximum rate. As interpreted, this represents the interval during which all muscular tissue is contracting.

3. A tele-systolic phase (BC) lasting approximately 0.03 second during which the rate of contraction is progressively diminishing. During this stage the fractionate contractions of the more proximate portions are progressively converted into fractionate relaxations which oppose and tend to neutralize the fractionate contractions of the more distal portions of the tissue. This continues until an exact neutralization at the apex of the mechanical curve has taken place—a point which we term the end of mechanical contraction.

These three phases are followed by a phase lasting 0.03 second or less, during which the curve turns upward with a very gradual gradient owing to the fact that the fractional relaxations are beginning to predominate over the fractional contractions (CD).

If these interpretations prove correct then the apex of the mechanical contraction does not indicate the moment when the fractionate contractions have all ceased. At B some of the fractions of cardiac tissue have started to relax and to a distance beyond C other fractions con-

tinue to contract. Since the initiation of contraction at the proximal arm of the myocardiograph is indicated by the onset of the mechanical curve at A and the first evidence of relaxation at this point is indicated by a change of contour at B, it will probably be fairly accurate and allowable to estimate *the duration of the fractionate contraction* of the more proximal tissue from the period AB.

THE RELATION OF THE MYOGRAM TO AURICULAR SYSTOLE

The term "systole" (Greek, συστολή, a drawing together or shortening) is best defined by current usage as the period of mechanical shortening of the entire auricular musculature. So used, the term is evidently not synonymous with, but much longer than the fractionate contraction interval. The question remains: Does the myogram recorded from two points on an anterior auricular surface give the full systolic interval? It is obvious that the duration of the mechanical contraction recorded by the myogram will depend directly on the distance between the two points selected for its attachment. In other words, the shorter the distance between the two points selected, the more nearly the mechanical contraction recorded will approximate the fractionate contraction; the farther they are apart, the more nearly will the contraction correspond to the total auricular systole. It is obvious that when it is desirable to study the influence of nerves or chemicals on the functions of irritability and contractility, the former procedure is preferable; whereas, when it is desirable to make time comparisons with the systole of the auricle, the greatest possible distance should theoretically be chosen between the two approximating points. Following the evidence given by the spread of the excitation wave as established by Eyster and Meek (10) and Lewis, Meakins and White (11) the two points to be selected for obtaining the full auricular systole would be the sinus node and the tip of the auricular appendix. Unfortunately, however, it is not feasible in practice to record a reliable myogram from points lying in two different planes. The largest area that is feasible experimentally, consists of a stretch between the right border of the auricle and the auricular tip (maximum, 28 mm., in a large dog). Even when this is chosen, the curve is somewhat deformed by changes in the form of the auricular surface.

It is necessary, therefore, to determine (1) whether there exists an essential time difference between the mechanical contraction recorded from two near and two distant points; and (2) how much the first auricular activity precedes that recorded by the myogram.

In relation to the first question, experiments show that the end of mechanical contraction is reached distinctly sooner in curves recorded from points 3–4 mm. apart than in those recorded from those separated 25 mm. or more. On the other hand, no time difference, as regards the end of contraction, could be found between two myograms taken respectively from points separated 25 mm. and 5–7 mm. The only explanation suggesting itself for this is that in an area 5 mm. wide there is as good a balancing of contracting and relaxing fractions as over wider areas. Hence, nothing is gained by using points separated more than 10 mm., provided they are not selected on or toward the auricular appendage, for if this is done the onset is delayed.

The second question, as to how much the first mechanical activity of the auricle precedes the myogram curve, may be most satisfactorily answered by comparing the auricular myogram with the intraauricular pressure curve. This was recorded by the type of optical manometer previously described by the writer (12). The cannula of this instrument was inserted into the azygos vein and pushed into the auricle via the superior vena cava.

A study of such records (fig. 3, curve 4) shows that the intraauricular pressure begins to rise 0.014 to 0.033 second (average 0.022) before the myogram curve begins to descend (cf. table I). The conclusion is evident that *the myogram does not record the complete interval of auricular* systole. In the comparison of time relations with auricular systole, it is therefore necessary to record a simultaneous intraauricular pressure curve or somewhat less accurately add to this period of mechanical contraction of the myogram the average interval 0.022 second (see table 1).

Dynamic period of auricular systole. A further comparison of the myogram and the intraauricular pressure curve (fig. 3) shows that the pressure within the auricle does not continue to rise during the entire interval of auricular shortening but reaches its maximum or summit when the contraction curve has been only partly completed. Close inspection (cf. wave 4 of figure 3) shows that this maximum occurs approximately when the change in the gradient at B appears, i.e., where relaxation of some auricular muscle has already begun, although the resultant mechanical effect is still one of shortening. The duration of this rise averages 0.053 second (table I). It is apparent that only the early half of auricular systole is effective in producing a rise of intraauricular and presumably of intraventricular pressure. This may, therefore, be designated as the *dynamic period* of auricular systole.

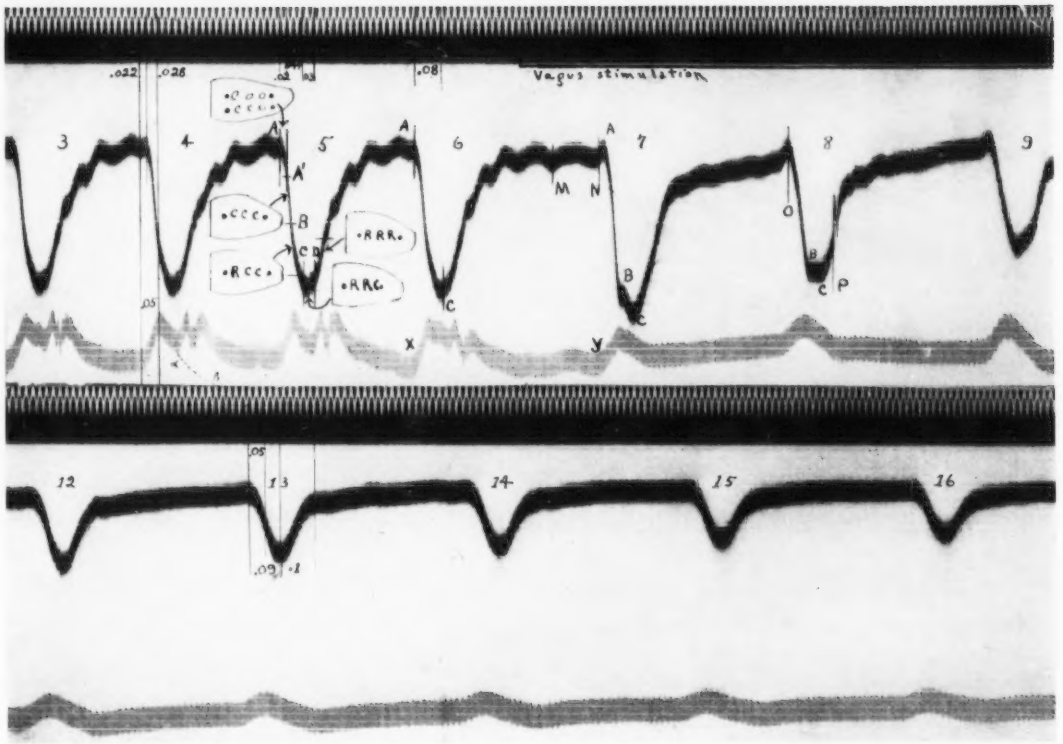

Fig. 3. Myogram of heart and synchronous intra-auricular pressure curve on rapid drum taken from points 12 mm. distant during normal action and during stimulation of left vagus. Waves are numbered from 3 to 16, in order to facilitate reference in text. Turning fork period equals 0.02 second. In the interpretative diagrams appended to wave 5, the two dots represent the points of attachment of the myocardiograph arms. *C* expresses a state of local contraction; *R*, relaxation; and *O* of rest.

Inasmuch as the dynamics of the heart beat are concerned with the maximum tension developed by auricular contraction rather than by its duration, this dynamic period and not the interval of auricular systole should be used when questions of dynamics are concerned.

When a ventricular contraction follows that of the auricle after a short interval, the fall of the auricular curve (extending into auricular diastole as sketched in at α β in wave 4 of figure 3 when the ventricle is not beating) is terminated by a sharp rise and fall due to ventricular activity. Its cause need not be considered here. Here, the entire rise and fall of the normal auricular wave within the auricle is completed during systole of the auricle. This confirms the results obtained recently by Ewing (18).

THE TIME RELATIONS OF AURICULAR EVENTS

The time relations of auricular events, as gathered from different experiments carried out under similar conditions are presented in table A. They show that the mechanical contraction recorded from the mid-auricular region or entire anterior surface of the auricle varies

TABLE A

EXPERIMENT	(1) FRACTIONATE CONTRACTION	(2) TOTAL MECHANICAL CONTRACTION	(3) INTERVAL BETWEEN RISE OF AURICULAR WAVE AND MYOGRAM	(4) TOTAL SYSTOLE (2+3)	(5) DYNAMIC SYSTOLIC INTERVAL
C75, VIII		0.070			
C76, IX	0.050	0.094			
C77, VI	0.062	0.093	0.033	0.126	0.038
C78, III	0.032	0.066	0.014	0.080	0 042
C77, IV		0.096	0.030	0.126	0.062
C80, XV	0.037	0.077	0	0.077	0.055
C81, VI	0.059	0.088			
C83, III		0.051			
C84, I		0.120	0.020	0.140	0.075
C88, I		0.078			0.050
C89, I		0.066			0.048
C90, III					0.060
C92	0.051	0.095			
C93	0.055	0.108	0.016	0.124	
C94		0.065			
C95	0.046	0.081			
C96	0.044	0.080	0.022	0.102	0.050
Average.....	0.0469	0.083	0.022	0.01107	0.0533

from 0.05 to 0.12 second, an average of 0.083 second. The interval from the onset of mechanical contraction to the second change in gradient, probably indicating the duration of fractionate contraction process, varied from 0.032 to 0.062 second, averaging 0.0469 second. The average of nine experiments shows that the intraauricular pressure curve precedes the myogram curve about 0.022 second, the shortest interval being 0.014, the longest 0.033 second. Adding these figures (or in cases where a simultaneous intraauricular curve was not taken, the average figure 0.022 second) it is found that the duration of the entire auricular systole averages 0.11 second, the widest variations being 0.077–0.140 second. The auricular wave of the intraauricular pressure curve reaches its maximum on an average within 0.053 second, the widest variation being from 0.038 to 0.075 second. Evidently the dynamic period of systole extends only over little more than the early half of auricular systole.

SUMMARY

1. The excitation wave, to judge from the appearance of negativity over different points of the auricle, spreads from the sinus node to more distant portions of auricular muscle. A short interval after each unit of cardiac muscle is so excited, it begins to contract. This local contraction, termed the *fractionate contraction* has an average duration of 0.469 seconds.

2. When the approximation of two points on the auricle is recorded by a myocardiograph, the myogram so obtained gives evidence in the changing rate of shortening that the onset of contraction develops at one point before it does at the other and spreads to the second attachment, within a period of approximately 0.02 second; after which the entire musculature between the points continues to shorten for 0.024 second. The remainder of the curve represents the resultant between the fractions continuing to contract and those that have already started to relax. This series of events represented by the mechanical contraction recorded by the myogram takes about 0.083 second. After a balance has been reached the two points begin to separate, at first slowly but later when all fibers have started to relax, more rapidly.

3. The interval of *mechanical contraction*, as established by the myogram, does not give the complete interval of auricular systole. This is evidenced by the fact that the intraauricular pressure curves rises

approximately, 0.022 second earlier. This interval added to the mechanical contraction, makes the average period of *systole* equal to 0.1107 second.

4. Auricular systole continues to develop tension, as indicated by the rise of the intraauricular pressure curve, only as long as all muscular units continue to contract. As soon as evidence appears in the myogram that the curve is a resultant of fractionate contraction and relaxation processes, the intraauricular pressure curve falls. The period during which tension is developed lasts about 0.053 second and may be designated as the *dynamic period of auricular systole.*

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NOTE ON PROTECTION OF STRING GALVANOMETER CIRCUITS AGAINST EXTERNAL ELECTRICAL DISTURBANCES

HORATIO B. WILLIAMS

From the Department of Physiology of Columbia University, New York

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The use of such an instrument as the string galvanometer which is capable of detecting very feeble electrical currents, even those of brief duration, is beset with special difficulties. Not the least of these is the difficulty of preventing disturbance of the instrument by adjacent electrical apparatus, motors, lighting circuits and the like. Not only is the galvanometer apt to be affected by the proximity of other electrical apparatus, but the electrostatic charges on rubber insulation, the clothing of the operator and other objects may become a source of trouble in a manner presently to be indicated. The disturbances that may arise from faulty insulation are perhaps more generally understood and will not be considered in this note. Granting that the insulation of the galvanometer circuit is adequate, external electric currents and charges can affect the instrument only by induction. We shall first consider

ELECTROMAGNETIC INDUCTION

Whenever a conductor which forms part of a closed circuit moves in a magnetic field in such a way as to vary the number of lines of force enclosed by the circuit, a current will be produced in the circuit. Also if a wire forming part of a closed circuit is so placed in a magnetic field as to enclose some of the lines of force, a variation of the strength of the field will alter the number of lines of force enclosed by the wire and a current will thus be set up in the wire though the wire itself is stationary. A few concrete examples may help to make clear the bearing of the above statements upon protection of galvanometer circuits. The wires which lead to and from the string terminals of a string galvanometer always pass through the relatively strong stray field of the instrument itself. If these wires quiver ever so slightly,

say with vibrations of the building or of motors used in connection with the apparatus, they will cut lines of force and set up currents through the string which become evident as a to and fro movement of its shadow having the same period as that of the vibrating wires. If any part of the string circuit runs near a wire transmitting alternating current for power or lighting, the variations in the magnetic field surrounding the power wire induce currents which cause the string to move to and fro with a frequency identical with that of the alternations of the power current. If a wire of the string circuit runs near a wire which supplies continuous current to the arc lamp usually used with string galvanometers, the string will move with every fluctuation of current in the lamp.

The easiest way to prevent trouble from movement of wires in the stray field of the instrument is to use very stiff wires and to fasten them so that the liability of movement is minimized. In other parts of the circuit the effect of varying fields, or of movement of the wires in steady fields, can be made very small by twisting together the (insulated) going and returning wires of the string circuit. Suppose the pole of a permanent magnet to be so moved that the number of lines of force in the region of an adjacent pair of wires is varied. If the wires are separated an appreciable distance they will enclose lines and the number of lines enclosed will vary as the magnet moves. According to the general statement made at the beginning of this section, a current in the wires will result. If, however, the wires are run close together so that very few lines can pass between them, then unless the field is very strong, the current produced by its variation, or by movement of the wire through it, will be feeble. If in addition the wires are twisted together, the spaces left between them will be a series of figures of "8" and a moment's reflection will show that the effect of a variation of the number of lines of force in one of the loops of the eight will be exactly compensated if the same variation occurs simultaneously in an adjacent loop. If a circuit is enclosed in an iron tube, magnetic lines external to it tend to flow in the wall of the tube on account of the great permeability of iron as compared with air. Only a very small number of lines will reach a circuit so enclosed. If the enclosed wires be also twisted, the protection will be still better. The thicker the iron wall, the more nearly complete will be the protection afforded. The protection is in no case absolute, but by sufficiently increasing the thickness of the armor it can be made as great as necessary. It is desirable that all power and lighting wires in the immediate neigh-

borhood of a string galvanometer equipment shall be enclosed in iron pipe and if such wires are also twisted together the magnetic effect of variable currents in them upon outside circuits will be further diminished. Wherever possible the outgoing and return wires of the string circuit should be twisted together and if necessary this circuit may be enclosed in iron also.

ELECTROSTATIC INDUCTION

If an insulated charged body is brought into the neighborhood of an insulated neutral body, an alteration of the distribution of electricity upon the latter occurs as illustrated in figure 1. While this change of distribution is taking place a momentary current will flow in the neutral body. If a second neutral body is brought into the neighborhood of the other two, there will be another change in the distribution of electricity on the first neutral body with another transient current, and so on. Consider the neutral body to represent the insulated wires, resistance boxes, switches, commutator, etc., connected to the string. Let the charged body be the hard rubber handle of one of the resistance boxes, charged by the friction of the operator's hand. Each increase or diminution of the charge will cause change in the distribution of electricity in the string circuit and the passage of a momentary current through the string. Once some part of the apparatus has acquired a charge, the mere movement of the operator's hand near the charged part is sufficient to disturb the electrical distribution and cause the passage of current through the string. Attention was directed to disturbances of this character by Einthoven in one of his earliest communications regarding the string galvanometer (1). Movement of any conductor near a charged body close to the string circuit will cause these disturbances. The conductor may be part of a piece of machinery, say a wheel connected with the apparatus for recording the movements of the string. In this case the disturbance will be rhythmic. It is unnecessary to multiply examples, as anyone who has worked much with these instruments will recall similar occurrences in his own experience. Not only do these disturbances occasion annoyance and sometimes lead to confusion

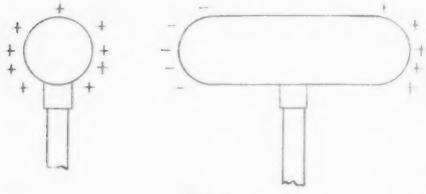

Fig. 1

in the interpretation of experimental results, but they are often of quite sufficient intensity to cause the loss of a string.

Electrostatic disturbances can be entirely eliminated by the method of "electrostatic screening." If an insulated conductor be completely enclosed in a hollow conductor which is at zero potential, the conductor within is entirely unaffected by variable or moving charges external to the hollow conductor.[1]

The hollow conductor at zero potential is called an electrostatic screen. For practical purposes it is not necessary that the hollow conductor be everywhere continuous. A wire mesh enclosing the apparatus to be protected is nearly as effective as a solid sheet of metal. The condition of zero potential is approximately satisfied by connecting the screen to the earth. In the practical application of the method to a galvanometer circuit it is customary to ensheath the wires of the string circuit in lead. Lead covered insulated wires are readily obtainable from dealers in electrical supplies in the larger cities. Such wire has the further advantage that it is so stiff and inelastic as to minimize the difficulties mentioned in the first section as arising from vibration of wires in an adjacent magnetic field. Rheostats and switches may be enclosed in boxes of sheet tin and the rheostats which have rotary contacts may be covered with wire gauze provided with perforations for the handles. The latter if of hard rubber may be covered with tinfoil. Plug rheostats are less easy to protect and inasmuch as boxes with rotary contacts can now be obtained of excellent quality at small expense, it is better to select them for this service. All the tin boxes, wire gauze, lead coverings of wires and the like should be earthed, preferably all to the same point. Usually connection to a water main gives a sufficient earth. Care should be taken that the connection is substantial as it is likely to become loose or corroded in time if made by merely twisting a wire about the pipe. A clamp is better.

NEW APPARATUS FOR COMPENSATION AND STANDARDIZATION.

The writer has had frequent opportunities to observe the inconvenience and inadequacy of apparatus supplied for compensation and standardization by the makers of string galvanometers. Most of these instruments are needlessly expensive and in many of them effective screening would be quite impossible.

[1] For the mathematical proof see J. J. Thomson, Elements of the mathematical theory of electricity and magnetism, 3d ed., 1904, p. 51-52.

Figures 2 and 3 are reproduced from photographs of an apparatus which has been developed by the Leeds and Northrup Company of Philadelphia to meet the writer's specifications for an adequate outfit of resistance boxes to be used in connection with a string galvanometer. It has been arranged primarily to meet the requirements of those who use the galvanometer for the clinical study of the heart, but will be found suitable for nearly every necessity which attends the use of the galvanometer in the physiological laboratory. The general arrangement is similar to that of the apparatus which has been in use for several years in the physiological laboratory of Columbia University and does not differ greatly from the scheme published by Einthoven (2). The noteworthy feature of the apparatus is that all the rheostats, switches and commutator are controlled by rotary handles and that coils and contacts are completely enclosed in a copper-lined box. All the handles are of metal which is in metallic connection with the copper lining and by connecting to earth the terminal marked "Earth," the entire apparatus is effectively screened. The binding posts for making connection to other apparatus can not be covered without difficulty, but they are placed at the back of the box, away from the region where the operator is likely to move his hands. One of these boxes was connected to a string galvanometer and the earth terminal purposely left disconnected. A light rubbing of the hard rubber top of the box with the finger was sufficient to cause the string to deflect so far that it came in contact with the rear wall of the space in which it moves and remained fast there until cautiously dislodged. After connecting the earth terminal to earth the top of the box was polished with a piece of silk without causing the slightest movement of the string.

The principle of the apparatus is illustrated in figure 4. An ordinary dry cell is connected in series with a resistance, R_1 which consists of a fixed resistance of 10,000 ohms and sufficient variable resistance to permit of adjusting the current which the cell will drive through, to the value 0.0001 ampere. The sensitive galvanometer, G, enables one to know when this amount of current is flowing. The resistance R_2 forms a derived circuit and when the current in R_1 has the value 0.0001 ampere, there will be a difference of potential of 0.001 volt across the terminals of R_2 for every 10 ohms introduced into R_2. This last statement requires the qualification that it is very nearly true unless the amount of resistance introduced into R_2 is an appreciable fraction of the resistance of R_1. In that case the current in R_1 will depart sensibly from the value 0.0001 ampere, but it can always be brought back to

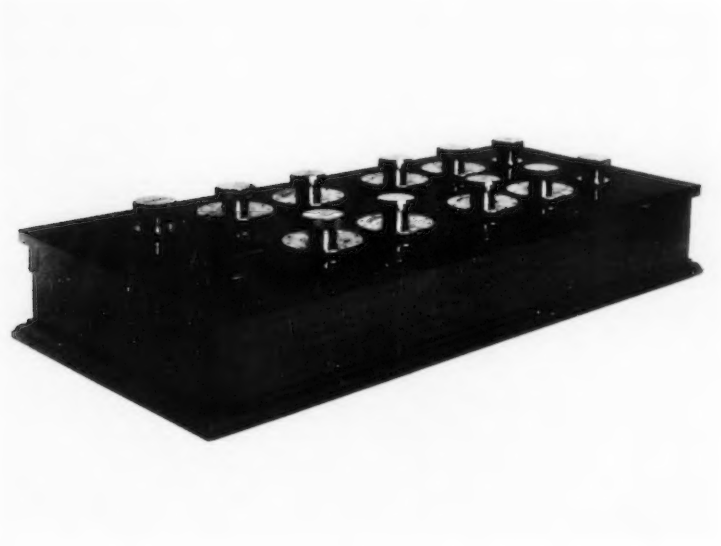

Fig. 2

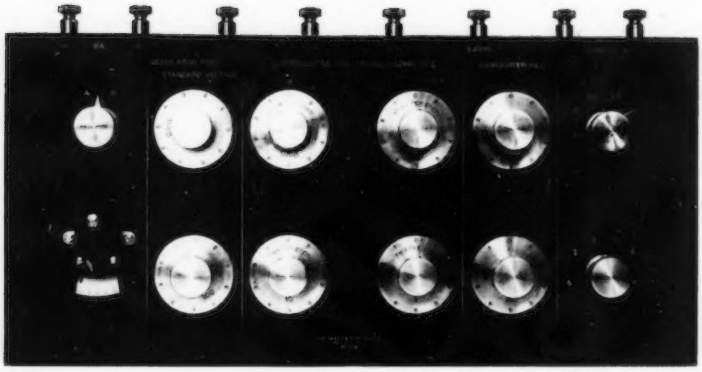

Fig. 3

that value by readjustment of the variable part of R_1 so that the standardization can be kept as accurate as the accuracy of the small galvanometer, G. This has an accuracy of 1 per cent. The greatest accuracy attainable in work with the string galvanometer is not more than 2 per cent, so that the galvanometer error is within the limit.

Referring to figure 3, the knob at the upper left hand corner marked "AB" is a commutator for the standard current. Below it is the galvanometer, "G." The two knobs marked, "Regulator for standard voltage," control the variable part of R_1. The four knobs marked "Compensating and standardizing resistance" control the resistance R_2. The terminals marked "$R.A.$" $L.A.$" etc., are for connection to the

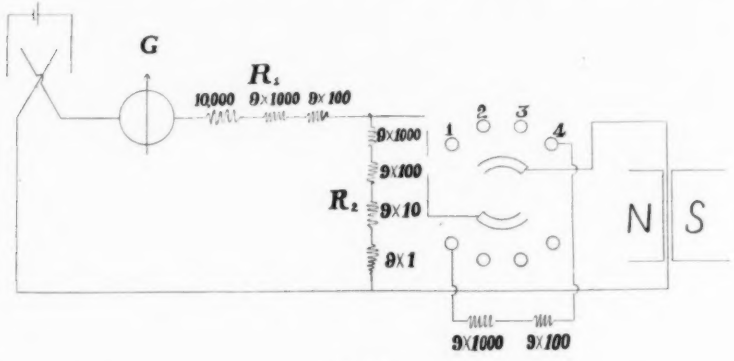

Fig. 4

extremities of the patient for clinical work and may naturally be connected in any convenient manner for laboratory purposes. In the lower right corner is a knob with a pointer and a series of figures, 1, 2, 3, 4. When the pointer is at 1, the galvanometer will be connected to the terminals $R.\ A.$ and $L.\ A.$, when at 2, to $R.\ A.$ and $L.\ L.$ following the usual manner of numbering the leads adopted by Einthoven for study of the heart. When the pointer is at 4, the galvanometer is connected to the resistance marked "Comparison resistance" which may be used to measure by substitution the resistance of a patient or physiological preparation. At the upper right corner is a knob which controls a resistance of 100,000 ohms and 10,000 ohms which may be put in series with the string during compensation to diminish the deflection.[2] The position for this knob when the instrument is out of use is at the point

[2] Not indicated in figure 4.

marked "Inf." This disconnects the galvanometer entirely and prevents the large throw of the string when the field magnet circuit is broken which is so apt to destroy strings in apparatus where a shunt resistance is used to cut down the sensitiveness during compensation.

SUMMARY

Methods of protecting string galvanometer circuits against electromagnetic and electrostatic disturbances are discussed and a brief description appended of an arrangement of resistances for use with string galvanometers in which effective electrostatic screening has been provided in the construction.

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ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION

I. The Action of Potassium Cyanide

L. H. HYMAN

From the Hull Zoological Laboratory, University of Chicago

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This paper is the first of a series intending to deal with the effect of a number of substances, especially anaesthetics, on the rate of oxygen consumption. My interest in this problem was aroused through the use of such substances as experimental agents for altering the rate of metabolic processes, for demonstrating the existence of metabolic gradients in organisms, and in the control of morphogenesis.[1] The present paper deals entirely with the action of potassium cyanide, a substance which has proved of peculiar value for the above purposes. It is hoped in the future to test similarly the action of alcohols, ether, chloroform, urethanes, acids, alkalies, and some salts.

A number of experiments have already been performed to determine the effect of cyanogen compounds on living matter. The classical experiments were those of Geppert (1), who in 1889 showed that the expired air of mammals under hydrocyanic acid poisoning contains more oxygen than normally, and, further, that the venous blood has a higher oxygen content than under normal conditions. From these results, Geppert concluded that cyanogen compounds act by reducing the capacity of the cells for consuming oxygen. Since these experiments it has been widely accepted that the cyanides produce their effect on protoplasm by directly depressing the oxidation processes. Geppert's work is, however, open to two criticisms. In the first place, it was long ago suggested that the cyanides may act by uniting with the oxygen-carrying compound of the blood, just as carbon monoxide does. Zeynek (2), however, has demonstrated that cyanides will not unite with haemoglobin at all, and with oxyhaemoglobin only after

[1] For a complete discussion of these matters, consult Child (5) and (6), where further references will be found.

heating several hours at body temperature. This criticism is, therefore, invalid. On the other hand, the work of Grove and Loevenhart (3) has shown that the action of cyanides in the case of mammals is primarily upon the respiratory center, and the reduced oxygen consumption observed by Geppert may be due in part to the general depression of the respiratory mechanism. Mammals are therefore unfavorable objects for this kind of experiment.

Nevertheless, subsequent work has entirely justified Geppert's conclusion. The best experiments that I know of are those of Schroeder (4) on the fungus Aspergillus niger; his data show a very marked decrease in both oxygen consumption and carbon dioxide output in the presence of rather strong concentrations of potassium cyanide. A number of other experiments have also been performed with cells, mainly egg cells, and with parts of animals;[2] but most of these have assumed rather than attempted to prove that the cyanides decrease oxygen consumption, although the results have always justified the assumption.

I thought it worth while to test directly the effect of potassium cyanide on some simple animal, since the only other direct measurements on animals have been performed on mammals; and to use a wider range of concentrations in order to determine whether or not very dilute solutions have a stimulating effect on oxygen consumption. The stimulating effect of potassium cyanide in very dilute concentration has already been noted by Lyon (7) who found an acceleration in the rate of development of the sea-urchin, and by Gasser and Loevenhart (8) who observed that the primary effect of cyanide on the medullary centers is a stimulating one.

The work was done at the Puget Sound Marine Station, Friday Harbor, Washington, where the University of Chicago kindly provided me with a research room.

MATERIAL AND METHODS

For these experiments it was thought advisable to select an animal which, first, has no muscular system, since variations in the degree of muscular activity are a source of error, and, secondly, no circulatory system, in order to avoid any possibility of an action of the cyanide on the oxygen-carrying compound of the blood. The only animals of sufficient size which fulfil these requirements are the sponges, al-

[2] References to this literature will be found in Child (5), p. 66.

though even they are capable of slight muscular activity. I found in the Friday Harbor region a heterocoelous calcareous sponge of the genus Suberites which proved well adapted for my purposes. These sponges were obtained by dredging at a depth of 60 to 90 meters, and were kept in a float car moored near the laboratory. The largest individual used measured 75 x 30 x 45 mm.; the others were somewhat smaller than this.

The sponge Suberites, unlike most sponges, is not attached to the bottom, but grows upon a shell inhabited by a hermit crab. It soon dissolves away the shell leaving within itself a spiral cavity which continues to be occupied by the crab. This sponge is therefore remarkably free from dirt and debris, and can be dredged without the usual injury attendant on separating a sponge from its substratum. For both of these reasons, Suberites seemed the most suitable of the available sponge material. The hermit crab must, of course, be removed.

The experimental procedure was very simple. Two wide-mouthed bottles, of about 1500 cc. capacity, were employed, one for a control, the other for the experiment. Each bottle was provided with a tightly fitting rubber stopper pierced by three glass tubes. Two of these tubes extended to the bottom of the bottle, one for the entrance, the other for the exit of water; the third tube extended only to the level of the stopper, and was used to admit air during the siphoning of the samples used for the oxygen determination. All connections were made as tight as possible.

The experiments were carried out on a raft fastened to the laboratory dock. Each experiment consisted of six half-hour determinations, three of normal respiration, and three of respiration in the presence of various concentrations of potassium cyanide. Fresh sea-water dipped up near the raft, and fresh cyanide, weighed out immediately before use, were employed for each of the half-hour determinations. The normal or cyanide-containing sea-water was siphoned simultaneously into both control and experimental bottles, the latter containing, of course, a sponge. The bottles were then suspended in the water at about a foot and a half below the surface (in order to secure a constant temperature). At the end of the half-hour period, they were drawn up, and a sample siphoned off from each into a small bottle, care being taken to secure a thorough sample by letting the bottle overflow for some time. These samples were then analyzed for oxygen content. A uniform order of procedure was adopted and maintained throughout all the experiments.

The oxygen content was determined by the Winkler method as given by Birge and Juday (9). Tests showed that the same quantities of the reagents may be used with salt as with fresh water. The thiosulphate was standardized with potassium permanganate instead of potassium bichromate because of the greater ease of determination of the end point.[3]

It is believed that all serious sources of error have been avoided. The principal precautions which were taken may be listed as follows:

1. The use of a control bottle of sea-water kept under the same conditions as the experimental bottle eliminates the possibility of error due to oxygen consumption by the numerous micro-organisms present in the water.

2. A practically constant temperature was maintained by immersing the bottles in the water. The temperature of the sea-water at the Friday Harbor station is very constant; during the four weeks occupied by the experiments, it varied between 11.2 and 13.7°C. The greatest variation recorded during the four hours required for each experiment was 0.9°, and a variation of this extent occurred only once. As these temperatures were necessarily read at the surface, it is certain that at the depth where the bottles were suspended the temperature varied even less than this.

3. The sea-water was shaken up vigorously before being used in order to secure a more uniform oxygen content. The oxygen content of the water near the laboratory varies greatly but for some unknown reason never attains the value which it should have at its temperature and salt content (according to the tables in Murray and Hjort (10)). The oxygen content of the shaken sea-water, in something over a hundred determinations, lay between 4.8 and 5.2 cc. per liter, being generally about 5 cc.

4. As regards the Winkler method, the chief criticism against it is the possibility of absorption of iodine by organic or inorganic substances (as H_2S) which may be present in the water; this would lower the oxygen value obtained. This source of error need not be con-

[3] To 100 cc. of distilled water add several grams of potassium iodide. (amount depends on the concentration of the standard solutions used). Acidify with a few drops of concentrated sulphuric acid and add a known, carefully measured amount of the standard permanganate. Titrate the iodine set free with the thiosulphate solution which is to be standardized using the usual starch indicator. This method is due to Prof. J. Stieglitz of the Kent Chemical Laboratory, University of Chicago. This laboratory also kindly furnished me with the standard permanganate used.

sidered here since it enters equally into the determinations in both normal and cyanide-containing sea-water, and does not affect the relative value of the results. Nevertheless, I have tested the iodine absorption of the sea-water directly by adding a small amount of standard iodine and titrating back with standard thiosulphate. In spite of the fact that considerable organic refuse is discharged into the water from the near-by salmon cannery, the iodine absorption of the sea-water is so extremely small as to be entirely negligible; nor do the sponges add any appreciable amount of iodine absorbing material to the sea-water.

5. In siphoning off the samples, it is necessary to admit atmospheric air. This may introduce an error as the water in the experimental bottle might take up oxygen during the siphoning, since its oxygen content has been reduced by the sponge. In order to minimize this source of error, the water was withdrawn from the very bottom of the bottles, the siphoning was conducted as rapidly as possible without any agitation of the bottles, and the siphoning was stopped when about half of the water in the bottles had been withdrawn. It is therefore highly improbable that any oxygen could have penetrated to the water which was siphoned off for analysis. Furthermore, this would be against rather than in favor of the purpose of the experiments as it would tend to reduce the difference between normal oxygen consumption and oxygen consumption in the presence of cyanide. The error could be avoided by having two experimental bottles each containing a sponge, and running the water from one into the other in siphoning. I tried this once or twice but the manipulation proved so cumbersome and the results so little different from the other method that I gave it up.

As a matter of fact, the chief difficulty encountered in the experiments was the behavior of the animals themselves, as will appear in the next section.

NORMAL OXYGEN CONSUMPTION OF THE SPONGES

It soon became evident that the normal oxygen consumption of any one individual sponge may vary greatly in successive half-hour determinations. This was an unexpected and undesirable state of affairs, since it would make the results with cyanide inconclusive. I discovered after a while that the cause of the variation in oxygen consumption lies in the condition of the oscula. Each sponge possesses on its upper side one to three large oscula, which are capable of contraction. When the sponge is stimulated, the oscula close slowly,

and remain closed for some time, after which they gradually relax again. In my experiments, the handling necessary in placing the sponge in the experimental bottle almost invariably brought about a closing of the oscula. The oscula then gradually opened until by the third half-hour, they were widely expanded, and remained so throughout the three following half-hour determinations.

When the oscula are closed, the oxygen consumption is diminished. The following data show this very clearly.

Experiment 14

	OXYGEN CONSUMED IN NORMAL SEA-WATER	CONDITION OF THE OSCULA
	cc.	
First half hour	0.54	Closed
Second half hour	0.85	Partly closed
Third half hour	1.22	Open

Experiment 15

First half hour	1.85	Open at beginning, closed at end
Second half hour	0.61	Closed
Third half hour	2.02	Open

Experiment 17

First half hour	1.64	Open
Second half hour	1.24	Partly closed
Third half hour	1.82	Open

Experiment 19

First half hour	0.52	Closed
Second half hour	2.09	Open
Third half hour	1.63	Open

Experiment 20

First half hour	0.29	Partly closed
Second half hour	1.19	Open
Third half hour	1.87	Open

Two possibilities suggest themselves to me as to the cause of the diminished oxygen consumption when the oscula are closed—either the activity of the cells of the sponge is diminished, or the decrease in oxygen content does not became apparent in the water in the bottle

because of the cessation of the water current through the sponge. In other words, the sponge may be using up the oxpgen in the water which fills its spaces, but since the oscula are closed, this water does not get to the outside. If this were the entire explanation, the apparent oxygen consumption when the oscula first open ought to be greater than it is later when the oscula have been expanded for some time. As this does not appear to be always the case, it is probable that both factors enter into the result.

The contractile oscula may be used for determining the irritability of the sponge by the familiar method of measuring the reaction time which elapses between the application of a stimulus, and the closure of the oscula. It was my intention to test in this way the effect of various concentrations of potassium cyanide on the irritability of the animals but owing to lack of time I was able to perform only a few rather hasty experiments. These indicated that the irritability is little if at all affected by the dilute concentrations but is considerably decreased in the strong concentrations of cyanide.

EFFECT OF POTASSIUM CYANIDE ON OXYGEN CONSUMPTION

The above observations show that it requires about one hour after the sponge is placed in the bottle for the oscula to resume their normal state of expansion. Although three successive determinations of normal oxygen consumption were made in each experiment, the first two have been discarded, and the figure for the third half-hour has been taken as representing the normal rate of oxygen consumption. It is this figure which appears in the tables below. Following this figure are three successive half-hour determinations of oxygen consumption in the presence of a certain concentration of potassium cyanide. As the importance of the condition of the oscula was not realized at first, no note of their state of expansion was made in the earlier experiments. In experiments 2 to 12, inclusive, it is not therefore certainly known that the oscula were open during the determinations but there can be but little doubt of this; in experiments 13 to 21, inclusive, I do know that the oscula were widely expanded throughout the four half-hours for which the figures are given. The data are arranged in the order of the concentration of cyanide used. The stated concentrations of the cyanide solutions are approximate only. The experiments starred were performed on the same individual sponge.

It should be added that complete recovery from the effect of the cyanide occurred in every case.

Experiment 5

	OXYGEN CONSUMED cc.
Half-hour in normal sea-water	0.72
First half-hour in 0.000005 mol. KCN	0.86
Second half-hour in 0.000005 mol. KCN	1.00
Third half-hour in 0.000005 mol. KCN	0.93

Experiment 3

Half-hour in normal sea-water	0.39
First half-hour in 0.00001 mol. KCN	0.41
Second half-hour in 0.00001 mol. KCN	0.23
Third half-hour in 0.00001 mol. KCN	0.16

Experiment 6

Half-hour in normal sea-water	0.88
First half-hour in 0.00001 mol. KCN	0.88
Second half-hour in 0.00001 mol. KCN	0.52
Third half-hour in 0.00001 mol. KCN	0.65

Experiment 11

Half-hour in normal sea-water	1.14
First half-hour in 0.00001 mol. KCN	1.14
Second half-hour in 0.00001 mol. KCN	1.32
Third half-hour in 0.00001 mol. KCN	1.16

Experiment 7

Half-hour in normal sea-water	0.39
First half-hour in 0.00002 mol. KCN	0.14
Second half-hour in 0.00002 mol. KCN	0.12
Third half-hour in 0.00002 mol. KCN	0.13

Experiment 8

Half-hour in normal sea-water	0.74
First half-hour in 0.00002 mol. KCN	0.80
Second half-hour in 0.00002 mol. KCN	0.66
Third half-hour in 0.00002 mol. KCN	0.12

Experiment 12

Half-hour in normal sea-water	1.26
First half-hour in 0.00002 KCN	1.30
Second half-hour in 0.00002 KCN	0.93
Third half-hour in 0.00002 KCN	0.87

*Experiment 13**

Half-hour in normal sea-water	1.55
First half-hour in 0.00002 KCN	1.72
Second half-hour in 0.00002 KCN	1.08
Third half-hour in 0.00002 KCN	0.99

Experiment 9

	OXYGEN CONSUMED c.c.
Half-hour in normal sea-water	0.93
First half-hour in 0.00004 mol. KCN	1.06
Second half-hour in 0.00004 mol. KCN	0.40
Third half-hour in 0.00004 mol. KCN	0.39

Experiment 14

Half-hour in normal sea-water	1.22
First half-hour in 0.00004 mol. KCN	1.09
Second half-hour in 0.00004 mol. KCN	0.88
Third half-hour in 0.00004 mol. KCN	0.69

*Experiment 15**

Half-hour in normal sea-water	2.02
First half-hour in 0.00004 mol. KCN	1.09
Second half-hour in 0.00004 mol. KCN	0.83
Third half-hour in 0.00004 mol. KCN	0.62

Experiment 2

Half-hour in normal sea-water	0.64
First half-hour in 0.0001 mol. KCN	0.14
Second half-hour in 0.0001 mol. KCN	0.18
Third half-hour in 0.0001 mol. KCN	0.14

Experiment 10

Half-hour in normal sea-water	0.98
First half-hour in 0.0001 mol. KCN	0.50
Second half-hour in 0.0001 mol. KCN	0.13
Third half-hour in 0.0001 mol. KCN	0.20

*Experiment 17**

Half-hour in normal sea-water	1.82
First half-hour in 0.0001 mol. KCN	0.61
Second half-hour in 0.0001 mol. KCN	0.57
Third half-hour in 0.0001 mol. KCN	0.43

Experiment 18

Half-hour in normal sea-water	0.78
First half-hour in 0.0002 mol. KCN	0.17
Second half-hour in 0.0002 mol. KCN	0.19
Third half-hour in 0.0002 mol. KCN	0.21

*Experiment 19**

Half-hour in normal sea-water	1.63
First half-hour in 0.0002 mol. KCN	0.46
Second half-hour in 0.0002 mol. KCN	0.21
Third half-hour in 0.0002 mol. KCN	0.13

*Experiment 20**

	OXYGEN CONSUMED cc.
Half-hour in normal sea-water	1.87
First half-hour in 0.001 mol. KCN	0.48
Second half-hour in 0.001 mol. KCN	0.16
Third half-hour in 0.001 mol. KCN	0.11

*Experiment 21**

Half-hour in normal sea-water	1.27
First half-hour in 0.001 mol. KCN	0.26
Second half-hour in 0.001 mol. KCN	0.22
Third half-hour in 0.001 mol. KCN	0.01

CONCLUSIONS

The following conclusions may be drawn from these experiments.

1. Very dilute concentrations of potassium cyanide increase the rate of oxygen consumption. This appears in experiments 5 and 11.

2. Slightly stronger concentrations, as 0.00001 and 0.00002 mol. have primarily a stimulating effect during the first half hour but later a depressing effect on the rate of oxygen consumption appears. This is the case in experiments 3, 6, 8, 12, 13, and 9.

3. In the stronger concentrations, 0.00004 to 0.001, the oxygen consumption is decreased throughout the entire period during which the animal was kept in the cyanide-containing sea-water. The decrease in oxygen consumption is very marked in the strongest concentrations used. Experiments 7, 14, 15, 2, 10, 17, 18, 19, 20, 21.

4. The effect of the cyanide varies with individual sponges. Thus the concentration 0.00004 may cause a primary stimulating effect followed by a depression in the case of one sponge, as in experiment 9, while in another sponge the same concentration shows only a depressing effect, as in experiment 14.

Attention may be called to the experiments which are starred, 13, 15, 17, 19, 20, and 21. These were performed upon the same individual sponge, the largest which I had. This sponge gave a high and fairly constant rate of normal oxygen consumption, and shows clearly the increasing depression accompanying increasing concentration of cyanide.

SUMMARY

The primary effect of potassium cyanide is a stimulation of the rate of oxygen consumption; this is followed by a depression of oxygen consumption which is the more marked the greater the concentration of

the cyanide used. Only in very dilute concentrations is the stimulating effect demonstrable; in stronger concentrations it is masked by the rapidly ensuing depression.

The use of potassium cyanide as an agent for depressing the oxidation processes is therefore entirely justified, provided the proper concentrations are employed.

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INCREASE OF PERMEABILITY TO WATER FOLLOWING NORMAL AND ARTIFICIAL ACTIVATION IN SEA-URCHIN EGGS

RALPH S. LILLIE

From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory, Clark University

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INTRODUCTORY

There are now many indications that the critical or initatory event in the activation of the resting egg is a temporary increase in the permeability of the protoplasmic surface-layer (or plasma-membrane) to water-soluble and diffusible substances. The precise connection between this variation of permeability and the total activation-process remains, however, to be determined. It is evident that in a system so complex as the egg-cell the immediate consequences of even so apparently simple a change may be various. The conditions of material interchange between egg and medium are altered; soluble substances, including possibly oxygen and carbon dioxide, enter and leave the egg more freely; there is also undoubtedly involved a change in the electrical polarization of the cell-surface, since the concentration and nature of the electrolytes on the two sides of the plasma membrane are altered. Only experimental evidence can decide finally as to the relative importance of these various possibilities. It seems likely, however, that the electrical change is itself the critical one. Electrical variations, due apparently to surface-changes, are well known to accompany stimulation and other cell-activities; and the general analogy between the initiation of division in resting egg-cells, and the excitation-process in irritable tissues, suggests that in the egg, as in the muscle-cell or nerve fiber, it is the electrical variation—and not the increase of permeability as such—which is the essential factor. External electrical currents stimulate muscles to contract; they may also activate the resting egg-cell in certain animals.[1]

[1] *Cf.* Schücking: Arch. f. d. ges. Physiol., 1903, xcvii, 86; Delage: Arch. d. Zoöl. expér. et gén., Ser. 4, ix, xxx; McClendon: this Journal, 1912, xxix, 299.

According to this view, activation as well as stimulation depends primarily on electrical conditions.[2] In most cases of activation, however, the change of permeability would appear to precede and determine the electrical change. We can thus understand how cytolytic substances, heat, mechanical injury to the cell-surface, as well as the electrical current, may induce either activation of the resting egg or stimulation. Cytolytic substances, which in general are parthenogenetic agents as Loeb has shown, have as a class a depolarizing effect on the cell-surface, as indicated by the currents of injury which they produce in muscle. Other activating agents (heat, mechanical conditions) cause similar effects. The chief facts showing a general connection between increase of permeability and activation are: (1) the increase of electrical conductivity following normal or parthenogenetic fertilization,[3] (2) the readier entrance of substances like alkali and dyes into the egg immediately after fertilization,[4] (3) the loss or secretion of materials from the egg soon after the entrance of the spermatozoon,[5] (4) the fact that pure isotonic solutions of neutral salts (NaCl, $NaNO_3$, NaI, KCN, etc.) which initiate cleavage in sea-urchin and starfish eggs, lose their activating power in the presence of calcium or magnesium salts or anaesthetics, which prevent their permeability-increasing action.[6] Facts of this kind have a more than special interest, since

[2] In several earlier papers I have urged the importance of the bioelectric processes in the activation of the resting egg and in cell-division: *cf.* Biol. Bull., 1909, xvii, 202 *seq.*; this Journal, 1910, xxvi, 116; Journ. Exper. Zoöl., 1913, xv, 25 *seq.* Bataillon (Arch. de. zoöl. expér. et gén., Ser. 5, 1910, vi, 128) also regards the electric variation as probably an important factor in activation.

[3] McClendon: this Journal, 1910, xxvii, 240; Gray: Journ. Mar. Biol. Assoc., 1913, x, 50.

[4] *Cf.* E. N. Harvey: Science, N. S., 1910, xxxii, 565; Lyon and Shackell: *ibid.*, 249.

[5] *E.g.*, exit of pigment in Arbacia eggs (Lyon and Shackell, *loc. cit.*); of other diffusible substances, probably chiefly salts, from Arbacia eggs (McClendon, *loc. cit.*, p. 264); similar phenomena in frogs' eggs (Backmann and Runnström: Arch. f. d. g. Physiol., 1912, exliv, 287). Phenomena of secretion are widespread; the separation of the fertilization-membrane probably belongs essentially in this category; decrease of volume with separation of materials from the egg is shown in vertebrate eggs (amphibia, fishes: *cf.* Bataillon, *loc. cit.*), and in many invertebrate eggs. The annelid egg affords striking instances, *e.g.*, Nereis (*cf.* F. R. Lillie: Journ. of Exper. Zoöl., 1912, xii, 414). The increased loss of catalase from the sea-urchin egg after fertilization, as observed by Lyon (this Journal, 1909, xxv, 199), is also probably a consequence of increased permeability.

[6] *Cf.* my papers in this Journal, 1911, xxvii, 289, and Journ. Exper. Zoöl., 1914, xvi, 591.

there is now little doubt that changes in the permeability and electrical polarization of the plasma-membrane constitute important controlling factors in a large variety of cell-processes. It is, therefore, desirable that our knowledge of these functional changes of permeability[7] should be extended in as many directions as possible.

During the last summer I have obtained definite evidence that the permeability of the egg to *water* undergoes marked increase immediately after fertilization. The entrance of water under the influence of osmotic pressure is several times more rapid in the fertilized than in the unfertilized egg. This may be shown readily as follows: Arbacia eggs are fertilized, preferably with the minimal quantity of spermatozoa, washed thoroughly with sea-water so as to remove the excess of sperm, and mixed with an equal quantity of unfertilized eggs. The mixed eggs are then transferred to hypotonic sea-water (*e.g.*, 40 volumes sea-water *plus* 60 tap-water), and examined at once under a low power. Within two or three minutes a well-marked difference in the size of the two kinds of eggs is apparent, the fertilized eggs being decidedly the larger. Measurement shows that after three minutes in the hypotonic medium the average diameter of unfertilized eggs is *ca.* 78 to 79 μ, that of fertilized eggs *ca.* 85 to 86 μ. Since the average diameter of the unaltered eggs in normal sea water is *ca.* 74 μ, the actual quantity of water entering the eggs in this interval of time may be estimated on the assumption that the eggs are spherical (volume equal to $\frac{4}{3}\ \pi r^3$). The volume of the normal egg of 74 μ is *ca.* 21.3 $\times$ 10^5 μ^3; that of the unfertilized water-distended egg of 78 μ is *ca.* 24.9 $\times$ 10^5 μ^3; of the fertilized water-distended egg of 85 μ is *ca.* 32.2 $\times$ 10^5 μ^3. The volume of water entering the unfertilized egg in three minutes is thus *ca.* 3.6 $\times$ 10^5 μ^3, that entering the fertilized egg is *ca.* 11 $\times$ 10^5 μ^3. This difference does not correspond exactly to the difference in permeability to water, as will be seen more fully below, but it illustrates clearly the greater readiness with which water enters the egg after fertilization. With a view to obtaining a more definite numerical estimate of the relative permeability of fertilized and unfertilized eggs, I have made a large number of measurements of the diameters of such eggs (and also of eggs with artificial fertilization-membranes) at different intervals after placing in dilute sea-water. From these measurements the rate of entrance of water—*i.e.*, of change of volume—can be determined.

[7] A general account of these phenomena is given in my paper in the Biol. Bull., *loc. cit.*, 199. *Cf.* also Bayliss: Principles of General Physiol., 1915, 124.

MEASUREMENTS OF EGGS IN NORMAL AND HYPOTONIC SEA-WATER

The diameters of the eggs were measured under a magnification of 480 diameters with an ocular micrometer, the divisions of which were standardized by a Zeiss stage-micrometer. The sea-water containing the eggs was mounted on an ordinary microscopic slide, which was fastened in a mechanical stage so as to admit of ready and accurate placing of the image of the egg over the scale. No cover glass was used; any distortion of the eggs by compression was thus avoided; the objective was immersed in the sea-water. Each division of the ocular micrometer corresponded to *ca.* 3.5 μ. The image of the egg is sufficiently sharp to admit of measurement within a fifth of a scale division. In transforming the readings to microns only the first decimal is to be regarded as significant.

As a rule Arbacia eggs are almost perfectly spherical in form; occasionally they are slightly ovoid. Only round eggs were chosen for measurement; otherwise the eggs were measured at random as they came into the field. A circular optical section is of course not a proof of spherical form, but evident departures from sphericity are not frequent in these eggs. Fertilized eggs, and especially eggs with artificial fertilization-membranes, are more likely than unfertilized eggs to become oval or otherwise deformed after some minutes in dilute sea-water. It is therefore especially important in measuring such eggs to choose those having a circular optical image.

Measurements in normal sea-water. The average diameter of 58 unfertilized eggs was 74.1 μ, with extreme variants of 71.9 μ and 75.7 μ. Fertilization appears to be followed by a slight decrease in volume,[8] due probably to secretion of a part of the cortical substance; 14 fertilized eggs measured within ten minutes after fertilization, showed an average diameter of 73.2 μ.

Measurements in dilute sea-water. All of the following measurements were made in a mixture of 60 volumes tap-water plus 40 volumes sea-water. In this medium (with an osmotic pressure of *ca.* 11 atmospheres) the eggs swell considerably, but osmotic disruption—when it occurs—does not usually take place for ten minutes or more. There is a striking difference between fertilized and unfertilized eggs in their resistance to this change. Of the two the fertilized eggs are much less readily broken down by osmotic distention;[9] in unfertilized eggs the entrance

[8] *Cf.* Otto Glaser: Biol. Bull., 1914, xxvi, 84.

[9] That is, previously to the appearance of the cleavage-furrow; at this time the membrane undergoes a marked change in its properties and is readily destroyed by osmotic distention.

of water is more gradual, but cytolysis always begins in a considerable proportion within fifteen minutes or even less in the solution, *i.e.*, some time before osmotic equilibrium is reached, and usually the majority of eggs are completely broken down within an hour. In the case of fertilized eggs water enters rapidly, osmotic equilibrium being usually reached within eight minutes or less, and the eggs remain intact without loss of pigment for several hours.

The volume of the eggs when osmotic equilibrium is reached is slightly less than double the volume in sea-water; the normal volume of the fertilized egg is *ca.* 20.6 $\times$ 10^5 cubic μ; in the above medium, after equilibrium is reached, it is *ca.* 40.4 $\times$ 10^5 cubic μ. If the egg were a perfect osmotic system (simple solution separated from external medium by a semi-permeable membrane) its volume would be inversely proportional to the osmotic pressure of the external medium; the sea-urchin egg approaches this condition closely enough to show that its behavior is essentially the same as that of an osmometer. It is to be expected that the egg will exhibit a smaller increment of volume in the hypotonic medium than would be the case with the ideal osmotic system, since a part of its volume consists of structural material, which occupies space but does not act osmotically.

The following tables (I, II) give measurements of the diameters of single eggs taken at regular intervals of one minute, begining one minute after placing in dilute sea-water. The procedure is simple; the normal eggs are kept in sea-water in finger-bowls; in each experiment the sea-water is first removed as far as possible, and a large volume (250 cc.) of the dilute sea-water is then added. The eggs are then mounted on the slide, and a single egg is chosen for observation; the necessary manipulation and the placing of the egg in position over the scale occupy some time, so that the first measurement cannot well be made sooner than one minute after placing in the dilute sea-water. Usually two eggs were kept under observation, and measurements were made, alternating from one to the other, at half-minute intervals.

Table I gives the diameters of three unfertilized eggs, from three different lots, measured at regular intervals of one minute up to fifteen minutes.

The degree and rate of water-intake shown by these measurements are typical. Seventeen similar measurements were made with unfertilized eggs; in six of these the eggs underwent cytolysis within eleven minutes or less; in nine cases regular measurements were made up to thirteen minutes or longer. In every case the egg showed the same

TABLE I

Diameters of unfertilized eggs in μ, measured at one minute intervals after placing in dilute sea-water (60 volumes tap-water plus 40 sea-water). (Average diameter of unfertilized eggs in sea-water, 74.1 μ)

LOT FROM WHICH EGGS WERE TAKEN	MINUTES AFTER PLACING IN DILUTE SEA-WATER														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A. (Sept. 1)	77.5	78.8	80.3	81.7	82.7	83.5	84.5	85.0	85.5	85.9	86.4	87.1	87.8	88.0	88.0
B. (Sept. 2)	75.7	77.4	78.8	79.3	82.0	83.8	84.5	84.8	85.2	85.5	86.2	86.9	88.0	88.5	89.0
D. (Sept. 7)	76.4	77.8	79.6	81.7	83.1	84.5	85.1	85.9	86.9	88.0	88.4	88.7	89.1	89.6	90.1

behavior; water enters gradually at a rate which changes only slightly during the first few minutes; and later falls off by degrees as the station of equilibrium is approached. Typically equilibrium is not yet reached by fifteen minutes, as the course of the curve indicates (fig. 1). Measurements of eggs taken from the general dish of dilute sea-water show that the intake of water continues for twenty minutes or more. The average diameter of eleven unfertilized eggs taken from different lots, measured after about eighteen minutes in dilute sea-water, was 89.9 μ.

Under the same conditions the initial entrance of water in fertilized eggs is several times more rapid than in unfertilized eggs, and osmotic equilibrium is reached within nine or ten minutes or even less. The same rapid rate of entrance is seen in eggs in which artificial fertilization-membranes have been formed by butyric acid (see curves, figs. 1 and 2). Table II gives measurements similar to those of Table I

TABLE II

A. Diameters of eggs fertilized with sperm ten to twenty minutes before placing in dilute sea-water. (Average diameter of fertilized eggs in sea-water, 73.2 μ)

EGGS FROM LOT	MINUTES AFTER PLACING IN DILUTE SEA-WATER												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. (Sept. 1)	82.7	84.5	86.2	88.0	89.2	89.8	89.9	90.8	91.5	91.5	91.9	91.7	91.9
B. (Sept. 2)	80.3	82.1	86.2	88.4	89.0	89.8	90.8	91.5	92.0	92.0	92.0	92.0	
C. (Sept. 3)	81.7	85.2	88.0	89.1	90.1	91.5	92.2	92.2	92.2	92.2	92.2		

B. Diameters of eggs in which artificial membranes were formed with n/260 butyric acid twenty to thirty minutes before placing in dilute sea-water.

D. (Sept. 7)	80.3	84.5	86.2	88.0	89.0	90.5	91.2	91.5	91.9	91.9	91.9	91.9	
E. (Sept. 7)	81.0	84.5	86.6	88.0	89.0	89.8	90.8	91.5	91.5	91.5	91.5	91.5	
F. (Sept. 7)	78.1	81.1	82.7	84.1	85.0	85.9	87.1	88.0	88.0	88.0	88.4	88.7	

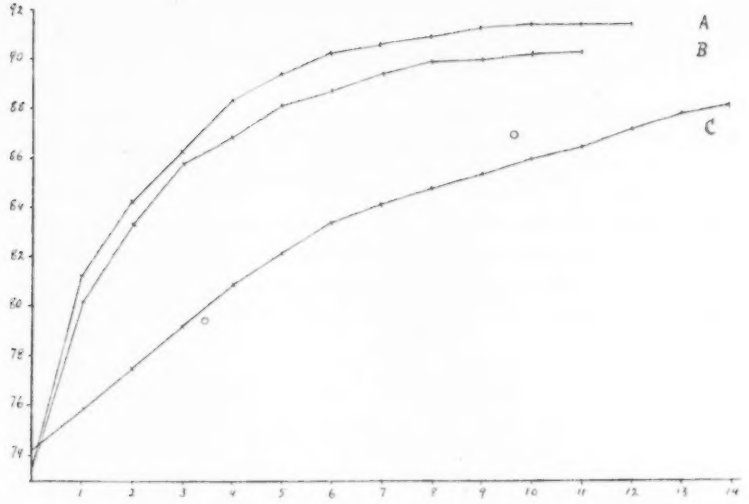

Fig. 1. Diameters of Arbacia eggs at different intervals after placing in dilute sea water. Ordinates are diameters in μ, abscissae minutes after placing in the hypotonic medium. The curves are not smoothed; the intersection-points of the co-ordinates are joined by straight lines. *A*, fertilized eggs; *B*, eggs with artificial membranes; *C*, unfertilized eggs.

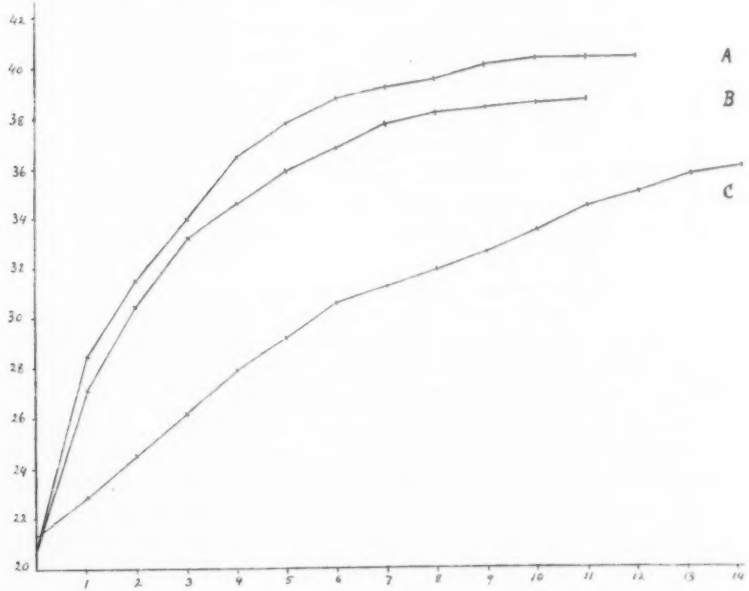

Fig. 2. Volumes of Arbacia eggs at different intervals after placing in dilute sea water. Ordinates are volumes (unit = 10^5 cubic μ); otherwise like figure 1.

for sperm-fertilized eggs and eggs with artificial fertilization-membranes. Two of the fertilized eggs and one of the eggs with artificial membranes came from the same lots (A, B, and D) as the unfertilized eggs of Table I.

The differences between the three kinds of eggs are indicated most clearly by the curves (figs. 1 and 2). The figures from which these curves are constructed represent the averages of a number of observations made on eggs from different lots. These averages are given in

TABLE III

Diameters and volumes of eggs at different times after placing in dilute sea-water

TIME	UNFERTILIZED (AVERAGE OF 4 EGGS)		FERTILIZED (AVERAGE OF 6 EGGS)		ARTIFICIAL MEMBRANES (AVERAGE OF 6 EGGS)	
	Diameters	Volumes (unit = 10^5 cubic μ)	Diameters	Volumes (10^5 cubic μ)	Diameters	Volumes (10^5 cubic μ)
minutes	μ		μ		μ	
0	74.1	21.3	73.2	20.6	73.2	20.6
1	75.9	22.9	81.3	28.4	80.2	27.1
2	77.5	24.5	84.3	31.6	83.3	30.5
3	79.2	26.1	86.3	33.9	85.8	33.2
4	80.9	27.9	88.5	36.5	86.9	34.6
5	82.2	29.2	89.5	37.8	88.2	36.0
6	83.5	30.7	90.4	38.8	88.8	36.8
7	84.2	31.3	90.7	39.3	89.5	37.8
8	84.8	32.0	91.0	39.6	90.0	38.3
9	85.4	32.7	91.4	40.1	90.1	38.5
10	86.1	33.6	91.5	40.35	90.3	38.7
11	86.6	34.6	91.5	40.35	90.4	38.8
12	87.3	35.1	91.5	40.35		
13	88.0	35.8	91.5			
14	88.4	36.3				
15	89.2	37.3				

Table III; this table includes the diameters of the eggs at the different intervals after placing in the dilute sea-water, and also the volumes (calculated on the assumption that the egg is spherical).

According to these measurements nearly five times as much water enters the fertilized as the unfertilized egg during the first minute after placing in the dilute sea-water, and nearly four times as much during the first two minutes. This difference, however, is not an exact measure of the initial difference in permeability to water, for the reason that the rate of entrance is not uniform, but falls off in a curve which

theoretically should have an exponential form, and more rapidly in the fertilized than in the unfertilized egg. What is required is the relative rates of entrance $\left(\frac{dv}{dt}\right)$ under the same conditions of osmotic pressure and area of membrane. From the above measurements, however, it is seen that during the first minute the fertilized egg takes up a volume of water of *ca.* 780,000 cubic μ, equal to 38 per cent of its initial volume; the egg with artificial membrane behaves similarly (*ca.* 700,000 cubic μ); while the unfertilized egg takes up only *ca.* 160,000 cubic μ,—*ca.* 7.5 per cent of its volume. This shows a somewhat remarkable resistance to the entrance of water, especially in the unfertilized egg. The surface-area of the Arbacia egg in sea-water is in round numbers *ca.* 17,000 square μ, and at the end of the first minute in dilute sea-water *ca.* 18,000 square μ. During the first minute therefore the volume of water transported across each square μ of surface is *ca.* 44 cubic μ for the fertilized egg, *ca.* 40 cubic μ for the egg with artificial membrane, and *ca.* 9 cubic μ for the unfertilized egg. This is with an initial driving force of *ca.* 13 atmospheres.[10] Later the difference between the rates of entrance is of course less; the slopes of the three curves indicate, as we should expect, that at osmotic equilibrium all eggs would have approximately the same volume,—about double that in normal sea-water; as already mentioned, however, unfertilized eggs frequently break down before this volume is reached.

The following table gives the observed average intake of water into the three kinds of eggs during the first ten minutes of immersion in dilute sea-water. It will be observed that in the unfertilized egg the average rate of entrance remains almost constant during the first six or seven minutes, but that in the other two it falls off rapidly from the

[10] The resistance encountered by water in penetrating the plasma membrane may be better appreciated if the facts are expressed in different units. One square centimeter = 10^8 square μ; one cubic centimeter = 10^{12} cubic μ; 9 cubic μ per square μ per minute is thus equivalent to 9×10^8 cubic μ per square centimeter per minute, or 0.0009 cubic cm. per minute. *I.e.*, approximately one cubic millimeter of water per minute per square centimeter of membrane, with a driving force of eleven atmospheres.

Harvey has remarked (*loc. cit.*, 565) upon the slowness with which Arbacia eggs swell in distilled water as indicating a surprising resistance to entrance of water. It is, however, clear that the surface of living cells must be highly resistent to the *solvent* action of water, and this implies resistance to its penetration. It would seem as if a membrane, in order to be truly semi-permeable, must have a limited penetrability to the solvent as well as to the solute. See below, p. 265.

first. This is a further indication of the difference in the rate at which water enters. The rate of entrance declines with the decline in the gradient of osmotic pressure between egg-contents and medium; this gradient decreases relatively gradually with the less permeable eggs. The curves (fig. 3) bring out this relation in graphic form.

TABLE IV

Average intake of water per minute in eggs (unit = 10^5 cubic μ)

PERIOD	FERTILIZED		UNFERTILIZED		ARTIFICIAL MEMBRANES	
	Total intake	Average per minute	Total intake	Average per minute	Total intake	Average per minute
minutes						
0–1	7.8	7.8	1.6	1.6	6.5	6.5
0–2	11.0	5.5	3.2	1.6	9.9	5.0
0–3	13.3	4.4	4.8	1.6	12.6	4.2
0–4	15.9	4.0	6.6	1.6	14.0	3.5
0–5	17.2	3.4	7.9	1.6	15.4	3.1
0–6	18.2	3.0	9.4	1.6	16.2	2.7
0–7	18.7	2.7	10.0	1.4	17.2	2.5
0–8	19.0	2.4	10.7	1.3	17.7	2.2
0–9	19.5	2.2	11.4	1.3	17.9	2.0
0–10	19.75	2.0	12.3	1.2	18.1	1.8
0–11			13.3	1.2		
0–12			13.8	1.15		
0–13			14.5	1.1		
0–14			15.0	1.1		

A more exact measure of the relative permeabilities is furnished by the relative times required for the entrance of the same volume of water. It will be seen from Table III that almost as much water enters the fertilized egg in one minute as enters the unfertilized egg in five; the egg with artificial membrane is only slightly less permeable than the fertilized egg. According to this comparison the plasma-membrane of the fertilized egg is from four to five times more permeable to water than that of the unfertilized egg. This conclusion is confirmed by the results of the analytical treatment which follows.

The rate at which water enters the egg—*i.e.*, the volume traversing the membrane in unit time,—is determined by several factors. Of these the chief are: (1) the driving force of osmosis; this (assuming that the membrane is semi-permeable) is at any time equal to the difference between the internal and the external osmotic pressures

(*i.e.*, *ca.* 13 atmospheres at first), diminished by the opposing forces of cohesion or elasticity of the egg-substance and the surface-tension; (2) the frictional resistance to the passage of water through the membrane (the reciprocal of the permeability to water);[11] and (3) the area of the membrane, *i.e.*, of the egg-surface. Two of these factors, (1) and (3), vary continuously as water enters and the volume of the egg increases; probably also the permeability of the membrane varies somewhat as its area is enlarged, but the comparatively regular course of the curve in the fertilized eggs indicates that during the period of

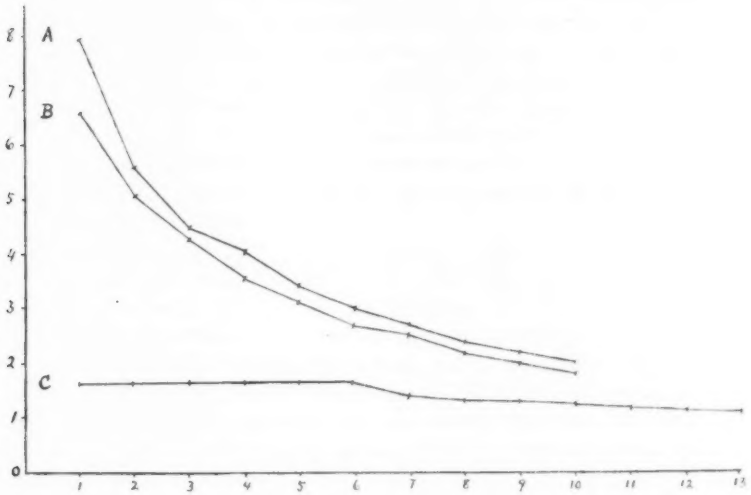

Fig. 3. Average intake of water per minute during immersion in dilute sea water for varying periods. Ordinates are volumes (unit = 10^3 cubic μ); abscissae, total time of exposure to dilute sea-water.

observation this change is relatively slight; in the unfertilized eggs the curve is less regular and its course for the first six or seven minutes is nearly straight; this appears to indicate that the decrease in the osmotic driving force during this period is nearly balanced by a slight though progressive falling off in the resistance to entrance to water. The calculated permeability-constant K does increase somewhat

[11] For a discussion of the rôle played by this factor in osmotic phenomena in general, *cf.* Antropoff: Zeitschr. f. physik. Chem., 1911, lxxvi, 721.

during this period (see Table V). On the whole, however, the alterations of permeability do not appear to be great, at least during the first ten minutes of immersion; later, when the extension of the membrane becomes sufficient to alter its structure, the general permeability must increase rapidly, as shown by the loss of pigment and general disintegration. In the following calculation I shall for simplicity leave out of consideration the forces of elasticity, cohesion, and surface-tension—which are undoubtedly negligible in comparison with osmotic pressure—and also the change in the area of the membrane during the period of water-intake. A calculation of permeability-constants on the basis of the volume of water passing across the *unit area* of membrane in each of the times considered (instead of that entering the whole egg) would be theoretically more exact; but it introduces complexities of treatment which it seems best to avoid, and does not alter the result in any essential manner.

According to the above analysis of conditions the rate of entrance of water into the egg at any moment will be indicated by the equation:

$$\frac{dv}{dt} = q\sigma\,(P_e - P_m) + \text{const.} \qquad (1)$$

where v is the volume of water entering in the time t, q the area of the membrane, σ the permeability of the membrane to water (reciprocal of frictional resistance to its passage), P_e the osmotic pressure of the egg-contents, P_m that of the external medium. *Const.* indicates any other factors, *e.g.*, physiological, which are additive in their effect.

The above equation is similar in form to that representing the course of a monomolecular chemical reaction, in which, according to Guldberg and Waage's law, the rate of change is at any moment proportional to the concentration (= osmotic pressure) of the substance undergoing transformation—*i.e.*: $\frac{dc}{dt} = Kc$, of which the integration-form is: $k = \frac{1}{t} ln \frac{a}{a-x}$, a being the initial concentration of the substance, and $a-x$ the concentration at the end of the time t. Similarly, assuming that q and σ in the above equation remain essentially unchanged during the period under consideration, the rate of entrance of water into the egg is at any moment proportional to the osmotic driving force, $P_e - P_m$ —*i.e.*:

$$\frac{dv}{dt} = K\,(P_e - P_m) + \text{const.} \tag{2}$$

where K is the constant indicating the rate of entrance of water into the egg under a definite pressure. This constant may be called the "permeability-constant." The integrated form will be:

$$K = \frac{1}{t}\, ln\, \frac{P_e - P_m}{P_t - P_m} \tag{3}$$

where P_e is the osmotic pressure within the egg at the beginning of the experiment (= the osmotic pressure of sea-water), P_m that of the external medium, and P_t the osmotic pressure within the egg at the end of the time t. It is evident that since the egg is originally in osmotic equilibrium with sea-water, the osmotic driving force at the outset of the experiment is the same as the difference between the osmotic pressures of sea-water and external medium. This osmotic force is $P_e - P_m$; after the time t, when the pressure within the egg has fallen to P_t, the osmotic force becomes $P_t - P_m$.

In order to apply this formula to the above observations it is necessary to translate the terms of osmotic pressure into those of volume. The measurements given in Table III show that the volume of the egg increases steadily, after placing in dilute sea-water, until osmotic equilibrium is reached; then the osmotic pressure inside the egg is equal to that outside. They also show that the ratio of the final to the initial volume is approximately the same as that of the initial to the final osmotic pressures within the egg (= the ratio of the osmotic pressures of sea-water and external medium); in other words, the volume of the egg, in a medium of about half the original osmotic pressure, is approximately doubled. If we regard the egg as a perfect osmotic system (with volumes the reciprocals of osmotic pressures), we may substitute volumes for pressures in the above equation; we may then compare the theoretical with the observed volumes. The equation then takes the form:

$$K = \frac{1}{t}\, ln\, \frac{V_{eq} - V_o}{V_{eq} - V_t} \tag{4}$$

Where V_{eq} is the volume of the egg at osmotic equilibrium in dilute sea-water, V_o its volume at the beginning of the experiment (*i.e.*, its volume in sea-water), and V_t its volume at the end of the time t. $V_{eq} - V_o$ thus represents the value of the osmotic driving force at the

beginning of the experiment, and $V_{eq} - V_t$ its value at the end of the time t.[12]

If the entrance of water in the foregoing experiments is in fact mainly determined by osmotic conditions, the value of K as deduced by this formula from the above observations should be approximately constant. In the following table K is evaluated for each of the three kinds of egg, substituting for V_{eq}, V_o, and V_t the volumes given in Table III. The final volume V_{eq} is regarded the same in all eggs, namely 40.4×10^5 cubic μ; the initial volume of the unfertilized egg is 21.3×10^5 cubic μ; that of the eggs with artificial membrane is assumed to be the same as that of the fertilized eggs (20.6×10^5 cubic μ). In the evaluation of K common logarithms are used.

In the unfertilized eggs K shows relatively little variation during the first fourteen minutes in dilute sea-water. In the fertilized egg the entrance of water during the first minute is always relatively large (See Table II). This is probably the expression of a disturbance (analogous to osmotic stimulation) due to sudden transfer to the dilute medium. During the next seven minutes K shows only minor fluctuations about a mean value which is about four times that of the unfertilized eggs. The approximate constancy of K shows that the entrance of water into the egg in dilute sea-water is determined by purely osmotic conditions. In the eggs with artificial membranes K is also three or four times greater than in the unfertilized eggs, and the initial rate of entrance is higher than later; in these eggs, however, K is not constant but shows a steady decline. This feature is interesting, since it indicates an imperfect semi-permeability and an inability of the membrane to resist extension; probably it is to be correlated with the fact that the condition of such eggs is unstable; normally

[12] The general solution of a problem of this kind, as given to me by Prof. A. G. Webster, is as follows:

$$V_t = V_o + (V_{eq} - V_o)\ (1 - e^{-kt}); \text{ hence:} \tag{1}$$

$$\frac{V_t - V_o}{V_{eq} - V_o} = 1 - e^{-kt}; \text{ that is:} \tag{2}$$

$$e^{-kt} = 1 - \frac{V_t - V_o}{V_{eq} - V_o} = \frac{V_{eq} - V_t}{V_{eq} - V_o}; \text{ or expressed in logarithms,} \tag{3}$$

$$-k = \frac{1}{t} \log \frac{V_{eq} - V_t}{V_{eq} - V_o}, \text{ or } k = \frac{1}{t} \log \frac{V_{eq} - V_o}{V_{eq} - V_t}. \tag{4}$$

TABLE V

A. Unfertilized eggs

(*Units of volume* = 10^5 *cubic* μ.) V_o (*initial volume*) = 21.3; V_{eq} (*final volume*) = 40.4; $V_{eq} - V_o = 19.1$

t (TIME IN MINUTES AFTER PLACING IN DILUTE SEA-WATER)	V_t (VOLUME AT TIME t)	$\frac{V_{eq} - V_o}{V_{eq} - V_t}$	$\frac{1}{t} \log \frac{V_{eq} - V_o}{V_{eq} - V_t} = K$
1	22.9	1.1	41
2	24.5	1.2	40
3	26.1	1.4	49
4	27.9	1.6	51
5	29.2	1.7	46
6	30.7	1.9	47
7	31.3	2.1	46
8	32.0	2.3	45
9	32.7	2.5	44
10	33.6	2.8	45
11	34.6	3.3	46
12	35.1	3.8	48
13	35.8	4.2	48
14	36.3	4.7	49
Average.....			46

B. Fertilized eggs

$V_o = 20.6$; $V_{eq} = 40.4$; $V_{eq} - V_o = 19.8$

1	28.4	1.6	204
2	31.6	2.25	171
3	33.9	3.0	159
4	36.5	5.1	177
5	37.8	7.6	176
6	38.8	12.4	182
7	39.3	18.0	179
8	39.6	27.4	174
9	40.1	66.0	202
Average.....			180

C. Eggs with artificial membranes

$V_o = 20.6$; $V_{eq} = 40.4$; $V_{eq} - V_o = 19.8$.

1	27.1	1.5	176
2	30.5	2.0	150
3	33.2	2.7	144
4	34.6	3.4	136
5	36.0	4.5	131
6	36.8	5.5	123
7	37.8	7.6	123
8	38.3	9.4	122
9	38.5	10.4	113
10	38.7	11.6	106
Average.....			132

they break down in sea-water unless subjected to further treatment (*e.g.*, with hypertonic sea-water).

The plasma membrane of the unfertilized egg is thus characterized by a low permeability to water. During the early period of distention in dilute sea-water this condition shows little change; later the semi-permeability appears to break down suddenly, with a resulting disintegration of the egg. In consequence of fertilization the permeability rapidly increases to about four times that of the unfertilized egg; the formation of artificial fertilization-membranes has a similar effect upon the egg; but such eggs are less capable than normally fertilized eggs of resisting distension without alteration of the osmotic properties of the membrane.[13]

These facts suggest certain more general possibilities, to which brief allusion may be made here. If the permeability to water varies so widely in different functional conditions of the cell, it is probable that the permeability to other substances, which also apparently pass the membrane readily at all times, may similarly vary. The chief of these are carbon dioxide and oxygen; it is possible that under certain conditions the membrane may be relatively impermeable to such substances. The egg previously to fertilization appears to be enclosed by a membrane which is waterproof to a relatively high degree; and it seems probable that the isolation thus resulting may account in part for the low rate of metabolism.[14] The possibility that the per-

[13] It might be expected that the difference in the osmotic properties of the egg before and after fertilization would be associated with a difference in the physical consistency and other properties of the surface-film. Such a difference might be demonstrated by micro-dissection. Dr. Chambers, whom I have consulted on this point, informs me that the unfertilized egg is less easily cut or torn with a needle than the fertilized egg, and that the sides of the tear show a greater tendency to fuse. This difference of behavior is suggestive in relation to the foregoing observations.

It might be objected that these observations indicate merely a difference in the tenacity or extensibility of the membrane, and not in its permeability as such—just as (*e.g.*) a rubber net and a steel net are equally *permeable*, though unequally extensible. Water would enter more rapidly the egg with the more extensible membrane. But any change in consistency and especially a change in density (*i.e.*, colloid content), would almost certainly involve a change in permeability; there is moreover good independent evidence of such a change (see introductory section). The present paper, however, is concerned not so much with the conditions as with the fact of increased permeability.

[14] Lyon and Shackell (*loc. cit.*) find, however, that unfertilized and fertilized eggs stained equally with methylene blue, and decolorized by reduction in a stream of hydrogen in an Engelmann chamber, regain the color, on readmission

meability to carbon dioxide may vary is not to be summarily rejected simply on the ground of the lipoid-solubility of this compound. Such a phenomenon as the increased output of carbon dioxide in nerves during stimulation[15]—a change unaccompanied by heat-production[16]—may be simply an expression of increased permeability, rather than of increased formation of this compound within the cell.

It should also be noted that this impermeability to water argues a high degree of density and insolubility in the surface-film. These properties, however, are retained only during life; they must therefore be an expression of cell-metabolism. One fundamental feature of metabolism is that a variety of surface-active materials of low water-solubility are continually being produced; apparently these gather in the surface-film, and are as continually being disintegrated or removed, *e.g.*, by oxidation. Only on some such hypothesis can we understand the remarkable fact that living cells, despite the water-soluble nature of most of the substances composing them, and the large surface-area which they expose to the solvent action of the medium, do not undergo solution, but preserve their semi-permeability and other properties intact.[17]

of air, at about the same rate. They are inclined to interpret this observation as indicating an equal permeability to oxygen in both eggs. In view of the much higher rate of oxidation in the fertilized eggs, and the presence of substances in the protoplasm which compete with the methylene blue for oxygen, we should rather expect, if the permeabilities were equal, that the slowly oxidising unfertilized egg would decolorize *more* rapidly than the rapidly oxidising fertilized egg. The observation may thus indicate a relatively rapid entrance of oxygen into the fertilized egg; unfortunately it is not decisive either way.

[15] Tashiro: Amer. Journ. Physiol., 1913, xxxii, p. 107.

[16] A. V. Hill: Journ. of Physiol., 1912, xliii, p. 433.

[17] One of the observations of Lyon and Shackell has an interesting bearing on the present problem. They found that iodine is taken up much more rapidly by unfertilized than by fertilized eggs (*loc. cit.*). If, as they suggest, the iodine is disposed of by the lipoids of the plasma membrane, this fact would indicate a greater content of lipoid or fatty substances in the plasma membrane of the unfertilized egg. It is thus possible that a relation exists between iodine-combining power and impermeability. The impregnation of the membrane with certain fats or lipoids—*e.g.*, with an unsaturated compound like cholesterol, which is also water-insoluble—would increase at the same time both its iodine-combining power and its impermeability to water. If these are the actual conditions in the egg, it would appear that fertilization leads to an accelerated removal or destruction of such compounds.

SUMMARY

1. The rate of entrance of water into fertilized Arbacia eggs in hypotonic sea-water of *ca.* 11 atmospheres osmotic pressure is approximately four times that into unfertilized eggs.

2. The rate of entrance at any time is determined by the osmotic pressure gradient (between egg and medium) prevailing at that time, and by the permeability of the plasma membrane to water. This permeability is therefore four times greater in the fertilized than in the unfertilized egg. The artificial formation of fertilization membranes (by butyric acid) is followed by a similar marked increase of permeability to water.

3. The osmotic properties of unfertilized and normally fertilized eggs remain approximately constant during the first eight or more minutes of immersion in dilute sea-water. On the other hand, eggs with artificial membranes show a progressive change in osmotic behavior, indicating probably a relatively unstable condition of the plasma membrane.

4. The essential constancy in the rate of entrance of water (relatively to the existing gradient of osmotic pressure) into fertilized and unfertilized eggs, during a period in which the water-content of the egg is almost doubled, shows that the difference between the two kinds of eggs is due not to a difference in the condition of the internal protoplasm, but simply to a difference in the resistance of the membrane to passage of water.

CARDIODYNAMICS IN HEART BLOCK AS AFFECTED BY AURICULAR SYSTOLE, AURICULAR FIBRILLATION AND STIMULATION OF THE VAGUS NERVE

ROBERT A. GESELL

From the Physiological Laboratory, Washington University Medical School, St. Louis, Mo.

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The purpose of this research is to study the relation of ventricular efficiency to ventricular filling and to analyze and correlate the various effects of auricular contraction on cardiodynamics.

It is commonly stated that the ventricles tend to empty themselves with each ventricular systole regardless of the initial ventricular volume obtaining. In this statement the function of auricular systole is suggested, that is, auricular systole should increase ventricular output in direct proportion to the increased ventricular filling resulting therefrom. But this statement is an observation rather than an explanation and in addition needs some modification, for the reverse holds with equal force, that is, the greater the initial ventricular volume, the less perfectly do the ventricles empty themselves. Unless each statement is carefully modified it would be more accurate to state that increased ventricular filling, within certain limits, increases ventricular output. Even when initial volume is small the ventricles fail to empty themselves completely with each ventricular systole. In view of this fact it is very significant that increased volume should increase ventricular output at all. It proves that increased initial ventricular volume per se is not the factor determining ventricular output but rather the secondary conditions arising from the increased volume.

A sudden increase of ventricular volume as produced by auricular systole means an increased initial length of ventricular fiber, an increased initial intraventricular tension, and an enhancing surface-volume relation, i.e., a greater increase of ventricular volume than ventricular surface.

All these factors should work toward greater ventricular efficiency. They are suggested as a result of work on the turtle's auricles, the

properties of which permit independent analysis of the factors of initial length of fiber and initial tension in relation to muscular contraction.[1]

We see from the foregoing that with other things constant, ventricular output does not necessarily vary in direct proportion to the degree of ventricular filling, and the reason is obvious. If ventricular output is not solely dependent on ventricular volume per se, which means that the ventricular muscle is not strong enough under all conditions to produce complete ventricular emptying, the increased ventricular output resulting from increased ventricular filling will depend on the degree to which the secondary enhancing factors are increased by increased volume.

With each auricular systole the enhancing effects of initial length, initial tension and surface-volume relation are increased simultaneously. The increased enhancing effect of each will depend on various conditions. In addition, these factors influence each other. It is difficult therefore to determine quantitatively the importance of each. All that can be hoped for is a general analysis of these factors, pointing out their interrelation and in a rough way their relative importance.

In a previous research on the mammalian heart,[2] in which the effectiveness of auricular contraction was varied, changes of venous and arterial pressures were used as indices of propulsion of blood by the ventricles, indicating whether the blood was accumulating on the venous or on the arterial side of the ventricles. Though changes of ventricular volume resulting from auricular systole and ventricular systole were not recorded, the enhancing effect of auricular systole on ventricular efficiency was ascribed in the main to the filling effect of auricular systole on the ventricles.

In the present research more definite information was obtained. The effects of auricular contraction were studied from a number of points of view. Records were made of auricular contraction, ventricular contraction, variations of length of ventricular fiber (which indirectly gives ventricular volume changes), intraventricular tension (which shows both initial and final tension), volume output, venous pressure and venous pulse.

To determine with any degree of exactness the relative importance of the many effects of auricular systole on ventricular efficiency, control of conditions and limitation of variable factors to a minimum is highly desirable.

[1] Gesell: This journal, 1916, xxxix, 239.
[2] Gesell: This journal, 1911, xxix, 32.

The use of a modified heart-lung[3,4] preparation with the heart in block offers excellent opportunities for cardiodynamic study. In such experiments rate of auricular and ventricular contractions, nature of auricular contraction, time relation of auricular systole to ventricular systole, venous pressure and capillary resistance are all under perfect control. To simplify description, the apparatus employed in these modified heart-lung experiments will be described under three headings: (1) venous system; (2) arterial system; (3) pneumatic blood pump.

I. Venous System (see fig. 1). This system was devised to supply the heart with blood at any desired constant venous pressure. In the main it consists of an upper (4) and lower (7) reservoir, and overflow (8, 9 and 12) and heart flow (11 and 15). The blood in it takes the following course: reaching the upper reservoir (4) through tube (1) it passes to the lower reservoir (7). From there the blood may go either to the heart via the heart flow (11 and 15), or back to the pump via the overflow (8, 9 and 12). The lower reservoir is a specially constructed double boiler, fitted with an overflow (9) and a heart flow (11 and 15) The reservoir proper is fixed within the outer jacket (10) which is filled with water maintained at the proper temperature with burner (16). A funnel (11) passes from the bottom of the reservoir through the outer jacket and connects with the heart flow (15). The blood is filtered through glass wool in the funnel.

The flow of the blood from the upper to the lower reservoir is regulated to insure a continuous flow over the overflow cut (8), thereby giving a constant venous pressure. It will be noted that this cut is made to accommodate large overflows without raising appreciably the venous pressure. The small notch which prevents damming back of solution by surface tension serves the same purpose. The upper reservoir has two uses. It prevents formation of froth in the lower reservoir and insures a more uniform flow of blood into the lower reservoir than could be obtained directly from the pump. The gauze filter (5) on tube (1) serves the same purpose.

The magnitude of venous pressure is regulated by the adjustable stand (3). The entire venous system is attached to rod (2) and moves as a whole when the venous pressure is altered.

[3] Martin: Croonian Lecture, Phil. Trans. Roy. Soc. London, 1883, clxxiv, 663.
[4] Knowlton and Starling: Journ. Physiol., 1912, xliv, 206.

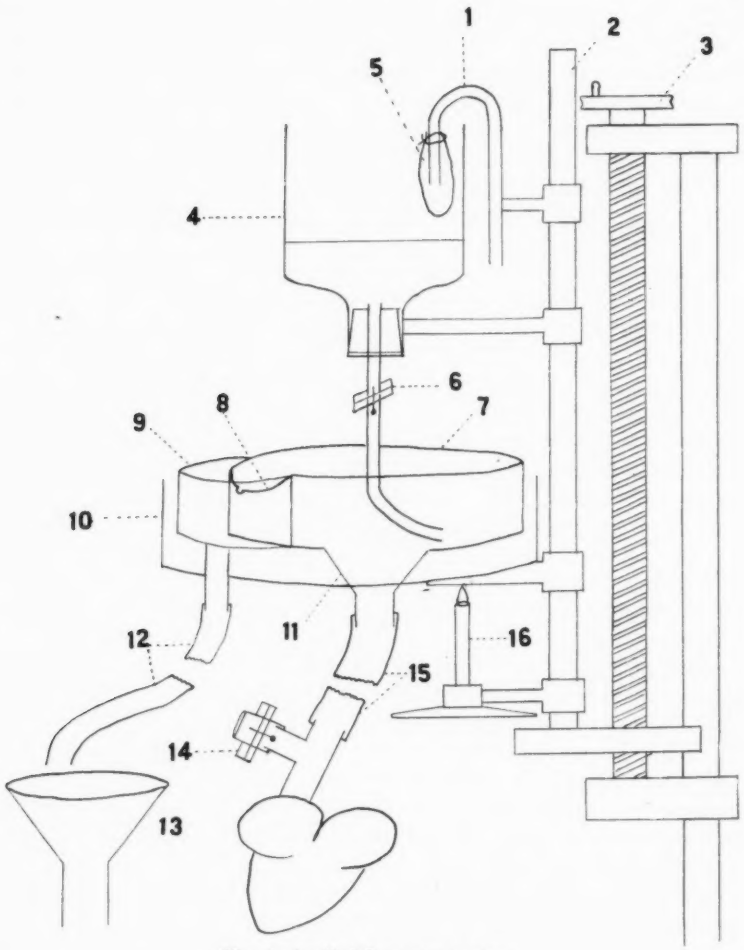

Fig. 1. Artificial venous system.

2. Arterial System (see fig. 2). This system is composed in the main of the capillary resistance (19 to 20), elasticity chamber (18), and differential volume flow recorder (34). As the blood comes from the heart it follows this route—through tube (17), into the capillary resistance (20), through (21 and 28) to the volume flow recorder (29,

30 and 34) and from there into funnel (13) back to the pump. The arrangement of the capillary resistance in the large T Tube (19) is shown. The pressure exerted on the outer surface of membrane (20) is regulated by the three way stopcock (33) which connects with the source of air pressure (22). With the cock in the position shown, the air pressure is transmitted to tube (19) and read off on mercury manometer (24). Air chamber (25) permits easy regulation of the pressure applied on membrane (20). Through tube (23) which is open to the exterior, the pressure may be decreased.

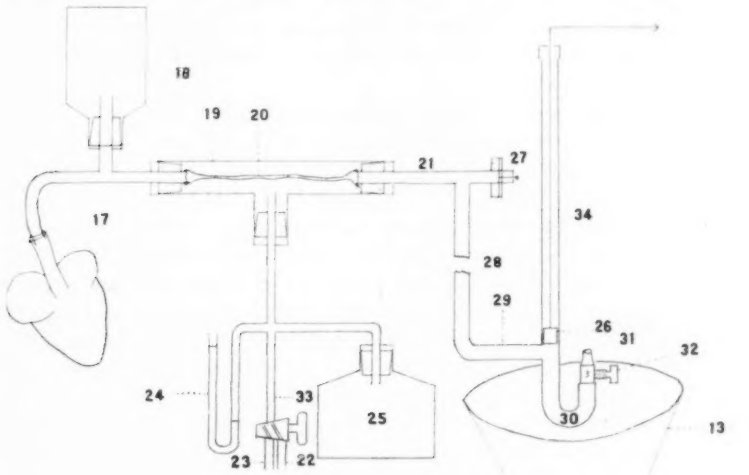

Fig. 2. Artificial arterial system.

Air chamber (18), as described by Knowlton and Starling, gives elasticity to the arterial system, permitting storage of ventricular energy.

A modified volume flow recorder similar to one previously described[5] was employed. It consists primarily of a three way connecting tube fitted with a graduated stopcock. The horizontal tube (29) is short and connects with the source of liquid to be measured. It joins at right angles a vertical tube (34) which contains a cork float and glass writing point (26). This tube connects with the U-tube (30) which is fitted with a graduated stopcock (32). The opening of this cock is on a level with the entering tube (29). This arrangement permits the

[5] Gesell: This journal, 1915, xxxviii, 402.

recording of flows from zero to a flow dependent on the height of tube (34) and the degree to which the cock is opened. This cock is threaded, and the block so marked that the degree of opening within one-fourth of a turn is easily read. The smaller the opening the more delicate is the recorder. A position of the cock suitable for an entire experiment is quickly found. Should a change be necessary the cock may be set at any known position and the recorder calibrated at the close of the experiment for the positions employed.

This recorder has certain advantages over such recorders as the tipping bucket, intermittent siphons, etc. It gives a continuous record, and consequently is quicker in indicating the moment of changed volume flow. It shows the pulse and gives an index to the individual ventricular outputs.

3. Pneumatic Blood Pump (see fig. 3). This consists of a system of valves in connection with a Woulfe's bottle which has three openings —blood outlet (46), blood inlet (47), and air inlet (48). The motive power is pulsatile air pressure furnished by the rotating stop cock (39) connected with the source of air pressure (40). A brass tube (36) passes through the blood inlet (47). This tube is closed at the lower end and its walls perforated with a number of holes which are covered on the outside with gold beater's skin (37). This arrangement permits blood to enter but not to leave the bottle through (36) and (47). The source of blood for the pump is the overflow and volume flow (12) and (34) reaching the blood inlet by funnel (13) and tube (35). The outflow tube (46) is closed above and open below, and nearly reaches the bottom of the bottle. The upper half of the tube (43) is enclosed by a larger tube (42) and its walls perforated and covered with gold-beater's skin (45). This arrangement directs the flow from the bottle through tube (1) into the venous reservoir and prevents backflow into the bottle. The capacity of the pump with a given air pressure depends upon the position of cock (38)—that is on the relative amount of air escaping and entering the bottle. The position of this valve and the rate of revolution of valve (39) can be so regulated that the pressure in the Woulfe's bottle falls to zero between each pulsation, permitting a free inflow of blood through the blood inlet.

OPERATIVE PROCEDURE

Prior to the experiment a large dog was bled, and the circulatory system washed out with Ringer's solution, until the volume of diluted blood obtained from the animal amounted to 1500 cc. The blood was defibrinated.

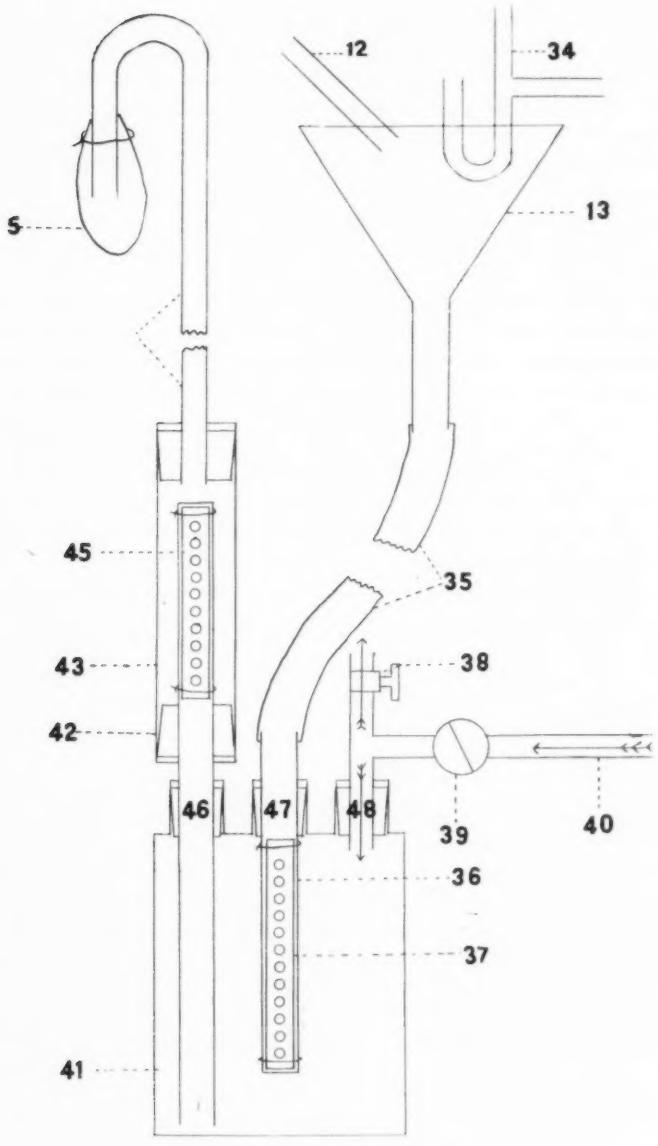

Fig. 3. Pneumatic blood pump.

The animal to be experimented upon was then prepared. Morphin and ether were given. The heart was exposed, and artificial respiration administered. A large cannula was inserted in the common carotid artery which was obstructed below the point of insertion. All the arteries springing from the arch were ligated and a heavy ligature passed under the aorta (but not tied) just distal to the common carotid. A large cannula (14) was then inserted into the superior vena cava and a heavy ligature passed under the inferior vena cava. The auriculoventricular bundle was crushed with the Erlanger heart clamp, and the clamp removed when block was complete. Arrangements were then made to record either arterial or left intraventricular pressure, as this is a necessary index to the working condition of the heart in the heart-lung preparation. The defibrinated blood, at body temperature, was set into circulation through the pump and venous system preparatory to perfusion. The blood circulating in the animal was then drawn in the following way. The obstruction below the arterial cannula was removed, the heavy aortic ligature tied, and the blood allowed to pass without resistance through (20) and out at (27), figure 2. This blood was defibrinated and used in the experiment. When the animal was bled the ligature on the inferior vena cava was tied, and the blood from the venous reservoir allowed to enter the heart through the superior vena cava. The resistance in (20) was immediately increased to the desired level. As Starling points out, the nutrition of the heart depends on the resistance offered to the blood. The success of the experiment, I have found, depends on promptly supplying sufficient venous pressure and arterial resistance. When once started the experiment goes on automatically for hours, without any further attention.

Since the circulation is confined to the heart and lungs only, the ether was now withdrawn. The auricular and ventricular contractions were recorded by the suspension and air transmission method. Intraventricular and arterial pressures were recorded with the Hürthle manometer, venous pressures by water and membrane manometers. The venous pressures were taken from the side tube of cannula (14) figure 2.

Intraventricular pressure was recorded with the aid of a trocar cannula previously used but not described.[6] Cannulas somewhat similar have been devised[7,8] but since the trocar cannula here employed has certain points of structure which may prove valuable to others it is

[6] Gesell: This journal 1911, xxix, 32.
[7] Straub: Arch. f. d. ges. Physiol., 1911, cxliii, 69.
[8] Piper: Arch. f. Anat. u. Physiol., 1912, 343; 1913, 385.

shown in figure 4 in its parts and assembled. It consists of a trocar (*A*), a cannula (*B*), a three way stopcock (*C*), and a sleeve with disc (*D*). The trocar (*A*) fits snugly in cannula (*B*) passing through and locking stopcock (*C*). The sleeve is threaded to fit the lower threaded end of the cannula. Before inserting and fixing the trocar cannula in position, it is assembled as shown with the sleeve turned high on the cannula. A purse string suture is stitched in the heart, and the trocar cannula passed through its center. The free ends of the suture are passed through a slit in the disc running from the periphery to the sleeve and tied about the sleeve. Holding sleeve and disc (*D*) the cannula is then turned so that the distance (*F*) equals approximately the thickness of the ventricular wall, insuring at all times the proper position of the intraventricular end of the cannula. Tube (*E*) is connected with the manometer, trocar (*A*) withdrawn to a marked point which unlocks the cock but still obstructs the upper end of the cannula; the cock is turned and the trocar completely withdrawn.

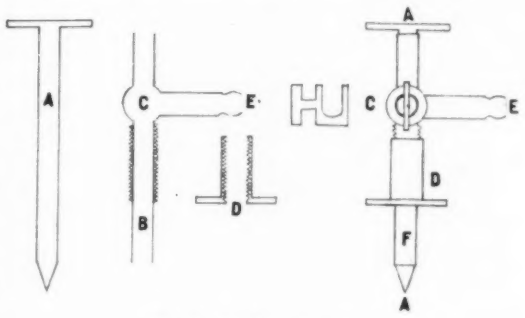

Fig. 4. Trocar cannula.

Changes in length of ventricular fiber were recorded and used as an index to ventricular volume changes, due to ventricular systole, filling action of venous pressure and auricular systole. The complexity of the experiment required a compact myocardiograph. The piston myocardiograph shown in figure 5 was employed. It consists of a cylinder and piston mounted on tubes (*A*), (*B*), and (*C*). The joint at (*A*) and (*B*) is welded; connecting (*A*) and (*C*) is a hinge joint permitting free movement in the plane of the piston stroke. The cylinder (*E*) is a thin turned brass tube, adjustable by block and set screw to any point on tube (*B*). Tube (*B*) is flattened on one side to fit a corresponding flattened surface on block (*F*), which prevents rotation of the cylinder on tube (*B*) thereby preventing binding of the piston. The piston myocardiograph is fastened in place by two threaded needles stitched into the heart, and the threads slipped under the needles and

tied as shown. The needles are inserted any desired distance into the tubes (*C*) and (*B*), and fixed with set screws (*G*). The device may be suspended by a light spring or thread; the best place of suspending the whole from tube (*A*) is soon found and fixed with colophonium. Cylinder (*E*) is then connected through tube (*K*) by rubber tubing with a piston recorder which records the changes of length of ventricular fiber.

In devising this piston myocardiograph effort was made to procure lightness, compactness, free movement and easy adjustment. The instrument does not interfere with the action of the heart and offers no difficulties if the piston recorder is properly balanced and freely moveable.

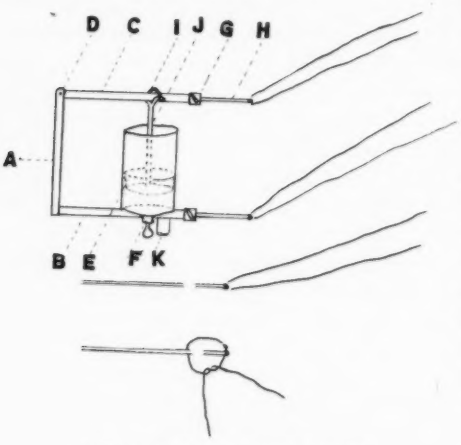

Fig. 5. Piston myocardiograph.

Time was marked in seconds and fifths of seconds.

The rate of ventricular contraction was controlled by unipolar stimulation selecting only the break shocks with a rotary stimulus selector. It was possible therefore, to study the effect of auricular systole on either slowly or rapidly beating ventricles.

The effects of auricular systole were studied by annulling or modifying the effectiveness of auricular contraction in various ways. In some cases the magnitude and rate of auricular contraction were controlled by faradic stimulation of the auricles and of the vagus nerve. In other cases the time relation of auricular to ventricular systole was changed, by the production of auriculo-ventricular interference waves (see fig. 6).

To produce these waves the ventricles are stimulated at approximately the rate of auricular contraction. The more closely the two rates are approximated the greater the number of cycles in the interference wave, and the more gradually does the time relation of auricular systole to ventricular systole shift. In figure 6 three types (*A*), (*B*), and (*C*) of interference waves are represented. In each type the upper

row of squares represents auricular cycles, the lower ventricular cycles. The black squares represent auricular and ventricular systoles respectively; the white squares auricular and ventricular diastoles.

Type (*B*) represents an interference wave in which auricular and ventricular rates are approximately equal. In this instance there are seven auricular to six ventricular cycles. Auricular systole (1) is completely stoppered by ventricular systole (1) and its effects therefore annulled. Auricular systole (3) occurs at the optimum moment, just completing at the onset of ventricular systole (3). The following auricular systoles shift back on the ventricular cycles until auricular systoles (7) and (1) are again completely stoppered by ventricular systoles (6) and (1) respectively. If auricular systole is important ventricular efficiency should be at its lowest at ventricular systoles (1) and (7), and at its highest at ventricular systole (3). Such is the case.

In addition to the type (*B*) two other types of interference waves, (*A*) and (*C*), proved of value in analyzing the effects of auricular systole. In type (*A*) the ventricular rate is approximately twice the auricular rate and in type (*C*) about half the rate.

Longer interference waves, with a greater number of auricular and ventricular cycles, permit minute and progressive changes of effectiveness of auricular systole and therefore offer exceptional opportunities for studying the effects of auricular contraction on ventricular efficiency.

Since magnitude of venous pressure might influence the relative importance of auricular systole this point was studied by varying venous pressure while interference waves were occurring.

The relative filling effects of venous pressure and auricular systole might vary in the case of the thin walled right ventricle and the thick walled left ventricle. With this point in mind, simultaneous records of right and left ventricular tension were made in several experiments.

RESULTS

Since the production of auriculo-ventricular interference waves permits either annullment of the function of auricular systole or the placing of auricular systole in its most effective position in ventricular cycle, this method gives maximum value to the importance of auricular systole for ventricular efficiency. Records obtained in the course of such waves are shown in figs. 7, 15, 16 and 17. Data obtained from waves under varying conditions are given in Table I. The

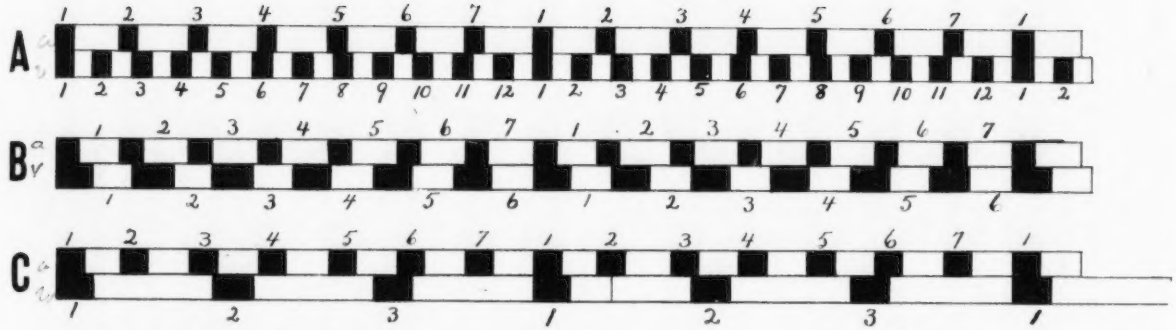

Fig. 6. Types of interference waves. *a*, Ventricular rhythm approximately twice the ~~ventricular~~ auricular rhythm; *b*, auricular and ventricular rhythm approximately equal. *c*, ventricular rhythm about half the auricular rhythm. The black blocks represent auricular and ventricular systoles, the white, diastoles.

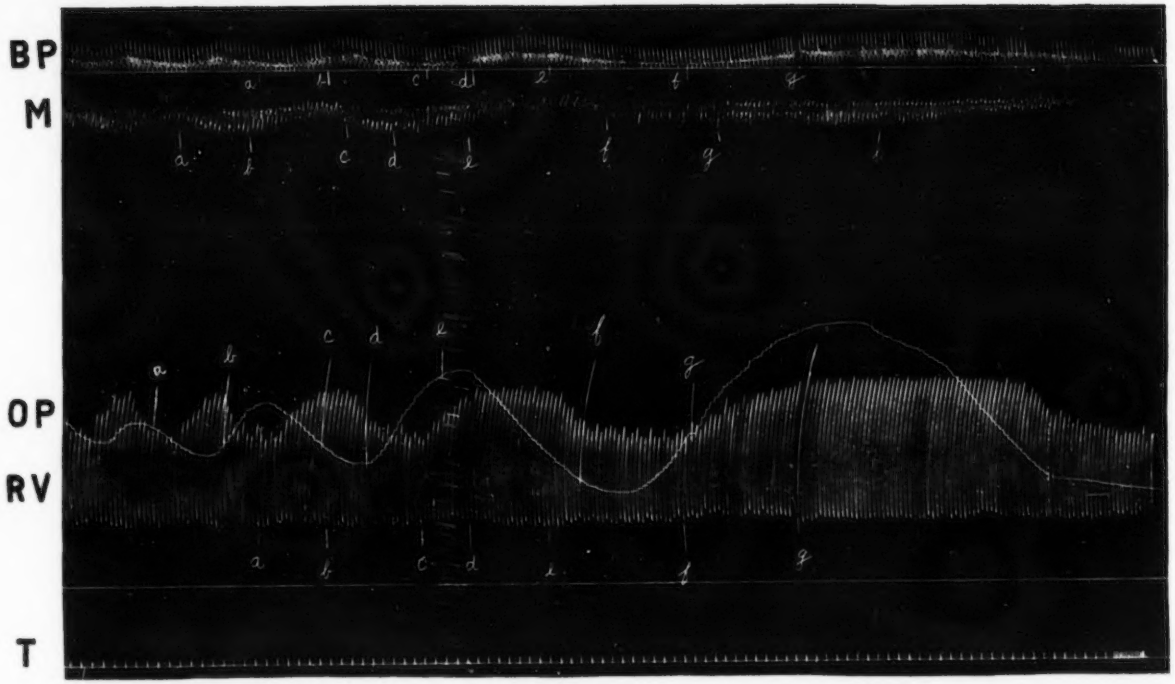

Fig. 7. Interference waves of various lengths showing fluctuation of initial and final volume length. Corresponding points are marked. *B.P.*, Arterial blood pressure recorded with the Hg. manometer; *M.*, myocardiograph tracing; *O.P.*, volume output; *R.V.*, right intraventricular pressure; *T.*, time in seconds.

rhythmical changes, due to varying effectiveness of auricular systole, on the auricular and ventricular suspension tracings, the myocardiograph, venous pulse, volume output, intraventricular and arterial tension tracings all give evidence of the influence of auricular contraction and permit the study of auricular contraction from several points of view.

Amplitude of auricular contraction, as represented by the auricular suspension tracing, is an index to the amount of blood propelled by auricular systole. If auricular systole occurs during ventricular diastole the auricular contents is readily passed into the ventricles and auricular amplitude is large. If auricular systole is stoppered by ventricular systole the amplitude is small. This is well shown in figure 17.

TABLE I

(1) TRACING	(2) VENOUS PRESSURE IN CM. OF BLOOD	(3) VENTRICULAR RATE PER MINUTE	(4a) (4b) MINIMUM AND MAXIMUM INTRAVENTRICULAR SYSTOLIC PRESSURE IN MM. Hg.		(5a) (5b) MINIMUM AND MAXIMUM VENTRICULAR OUTPUT IN CC. PER MINUTE		(6) PER CENT INCREASE OF VENTRICULAR OUTPUT
			Min.	Max.	Min.	Max.	
44	2.7	156	115	155	280	440	57
50	3.9	114	110	140	270	430	60
16	5.3	138	115	150	370	600	62
62	5.3	298	45	142	110	400	264
38	9.0	144	120	175	650	880	35

Intra-ventricular tension tracings show fluctuations of both initial and final tension resulting from altered auricular effectiveness. See figure 16. The volume output tracings show only indirectly the effects of auricular contraction.

Table I shows the minimum left ventricular systolic tension and volume output obtaining at the trough of an interference wave—at such a time, the tension and output are maintained by venous pressure alone. It also shows the tension and output obtaining at the crest of the same wave—the tension and output maintained by venous pressure and auricular systole of maximum effectiveness. The last column in the table gives the relative importance of auricular systole, that is the percentage increase of ventricular efficiency over that maintained by the filling action of venous pressure alone.

The interpretation of these results is the primary object of this research.

It has been suggested[9] that previous results obtained from interference waves might be adequately explained by variations of imperfection of valvular action. The possibility of disturbed valvular action accounting in part for the results obtained was pointed out at the time,[10] but was not considered important. But unless the degree of disturbed valvular action is determined, a careful analysis of other secondary factors is impossible. It, therefore, seemed advisable to study the relation of regurgitation to the results obtained.

Auricular systole immediately preceding ventricular systole may bring about better valve closure than venous pressure alone, thereby preventing regurgitation. But if the primary function of auricular systole is to insure perfect valve closure, amplitude of auricular systole presumably would have little effect on ventricular efficiency, provided auricular systole constantly precedes ventricular systole by the normal time interval. It has been shown in the case of the turtle's heart, that as the auricles undergo tonus oscillations, ventricular output varies directly as the amplitude of auricular systole. Though these experiments minimize the factor of disturbed valvular action in the mammalian experiments cited and emphasize the importance of ventricular filling, there might be some objection to applying the results directly to cardiodynamics in the mammalian heart. Valvular action was therefore further studied in the dog's heart. It seemed that the venous pulse would offer the best means for detecting the extent of regurgitation. This method was used, and in most instances a membrane manometer was employed to record the venous pressures.

The first experiments were planned with the object of determining whether auricular systole is necessary for perfect valvular action. This was done by stimulating the vagus nerve in the course of an interference wave, see figure 8. (*A*) to (*B*) represents an interference wave of the type (*C*), figure 6, in which the auricular rate is approximately twice the ventricular rate. The beginnings of auricular systoles are set off on the venous pulse tracings by the upper vertical lines, ventricular systole by the lower lines. Note the varying height of the venous waves and that the highest waves occur during interference of auricular and ventricular systole. These high positive venous waves occurring during interference may have two causes; back pressure from stoppered auricular systoles, and regurgitation at the onset of ventricular systole. By inhibiting the auricles the first cause is removed and any positive

[9] Henderson and Johnson: Heart, 1912, iv, 69.

[10] Loc. cit.

waves must therefore have another explanation. During complete inhibition, positive waves synchronous with ventricular systole do occur, but are of too small amplitude and too slow formation to be accounted for by regurgitation from the powerful ventricular contraction. The waves probably are due to the usual negative and positive pressures obtaining during early ventricular diastole and late ventricular diastole just prior to auricular systole. Under the conditions given, auricular systole does not seem necessary to insure perfect valvular action.

The next question is: Can auricular systole abnormally placed in the ventricular cycle disturb valvular action and permit appreciable regurgitation? Such disturbance would be most apt to occur during partial interference of auricular and ventricular systoles. Such disturbance may be analyzed from two points of view; one in which ventricular systole is in progress at the onset of auricular systole and the other in which auricular systole is in progress at the onset of ven-

Fig. 8. Interference wave Type C. Fig. 6 followed by vagus stimulation. *A.*, Auricular contractions; *V.*, venous pulse. Auricular systoles are marked off on venous pulse above, ventricular systole below.

tricular systole, see figure 9. Here ventricular and auricular systoles are set off on the venous pulse obtained in the course of an interference wave of 15 auricular and 16 ventricular cycles. Ventricular systoles are enclosed in brackets above, auricular systoles in the short lines below. Examination of this record shows that in every case the positive wave follows auricular systole by a short interval of time, that the wave increases in size only when auricular and ventricular systole begin to interfere, and that the amplitude of the wave depends upon the extent of interference. Auricular systoles (12, 13, and 14) occurring during ventricular diastole, produce the smallest waves, while auricular systoles (4, 5 and 6) completely stoppered, produce the largest waves.

If ventricular systole is in progress at the onset of auricular systole the positive wave does not occur until the onset of auricular systole—indicating again that auricular systole is not essential to perfect valve closure, (see ventricular contractions 4, 5, 6 and 7). The positive waves in these instances are clearly of auricular origin.

But when auricular systole is in progress at the onset of ventricular systole, the opportunity for regurgitation is greater. Whether regurgitation occurs under such conditions is difficult to determine definitely for a positive wave would result the moment both systoles were in progress, whether regurgitation occurred or not. Even under these adverse conditions valvular action is not appreciably disturbed, (see auricular systoles 0, 1, 2, and 3, and 15 and 16). Since ventricular systole is more powerful than auricular systole, it might be expected that any appreciable regurgitation would have a marked effect on the venous pulse. Careful examination of auricular, myocardiograph and tension tracings, however, gives occasional indication that some regurgitation may occur in one or two ventricular cycles in the course of a single interference wave. But whether such occasional regurgitation occurs or not matters little with the interpretation of results obtained from interference waves as will be seen in the following section.

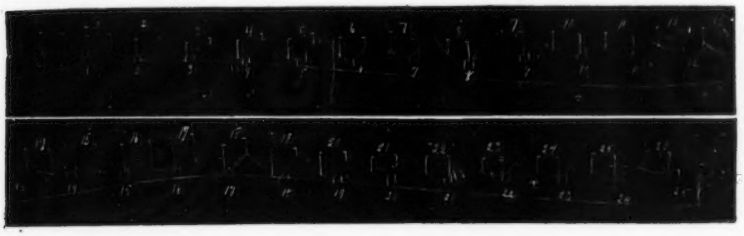

Fig. 9. Venous pulse tracing taken in the course of an interference wave. Veatricular systoles are set off above, auricular systoles below.

Interference Waves. We can gauge the extent of regurgitation by another method of analysis, namely, by noting the intraventricular pressure obtaining in various phases of interference waves. Two types of interference waves in which auricular and ventricular rates are closely approximated can be produced; one in which auricular rate is slower, and the other faster than ventricular rate. These two types are shown in figure 10. In the upper interference wave there are 21 auricular cycles to 22 ventricular cycles. In this type auricular systole shifts forward in ventricular cycle. In the lower wave there are 22 auricular and 21 ventricular cycles. In these waves auricular sytole shifts backward in ventricular cycle.

In each case, the upper blocks represent auricular cycles, the lower ventricular cycles. The black solid blocks represent auricular and ventricular diastoles.

Assuming that auricular systole has an enhancing effect on ventricular efficiency, and that this enhancing effect increases with the approximation of auricular systole to the normal position in ventricular cycle, and that regurgitation is not an important factor the curve of ventricular efficiency may be theoretically plotted for the two types of waves. The recorded intra-ventricular tension rather than the ventricular output is compared with the plotted curve, because the tension record is quickest to indicate changing ventricular efficiency. The shape of the efficiency curve varies in the two types, and is of value in the interpretation of results.

If auricular systoles are completely stoppered, venous pressure is the only filling force. This force is constant and, therefore, during this period, a constant level of ventricular efficiency should be maintained as shown by lines (AB) and (UV), as auricular systole advances or recedes the curve should follow two different courses. This course depends on a number of factors, the discussion of which would be too lengthy for this paper. Briefly stated, it depends primarily upon the duration of auricular systole and the relative duration of ventricular systole and ventricular diastole and whether auricular systole is advancing or receding in ventricular cycle. Ventricular diastole usually is considerably longer than ventricular systole and that relation is shown in the diagram. The only difference between the two waves is the direction in which auricular systole is shifting. In the upper wave auricular systole is advancing. The effect of auricular systole (3) is annulled. Auricular systole (18) is at its optimum position. In the 15 intervening cycles auricular systole gradually shifts from a position of complete annullment to that of maximum efficiency. Ventricular efficiency for this period is therefore represented by a gradual incline (BC). Following auricular systole (18) auricular systole shifts from a position of maximum to one of minimum efficiency in only 4 cycles as represented by the sudden drop to the horizontal (CA).

The plotted curve corresponds closely with the experimental results obtained, see figure 16.

In the lower wave the reverse conditions obtain. From the constant efficiency maintained by venous pressure (U to V) auricular systole shifts in 4 cycles (1 to 5) from minimum to maximum efficiency and in 16 cycles from maximum to minimum efficiency. The curve plotted corresponds approximately with the experimental results obtained.

In connection with the question of regurgitation the sudden drop of the curve (C to A) in the upper wave might in part be accounted for

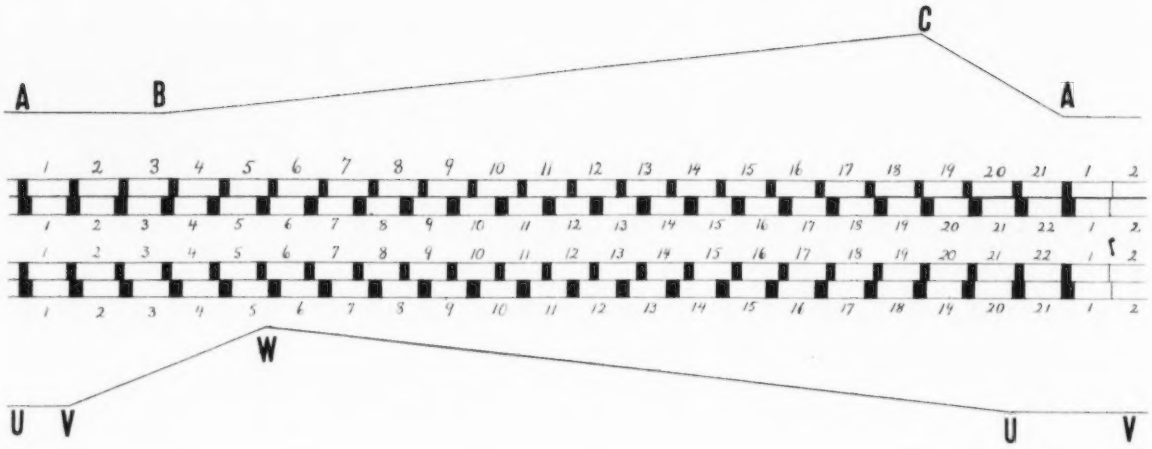

Fig. 10. Interference waves with auricular and ventricular rhythm approximately equal. Upper—21 auricular to 22 ventricular cycles. Lower—22 auricular to 21 ventricular cycles. Curves represent theoretically plotted ventricular efficiency.

by imperfect valve action; for here auricular and ventricular systole are most dangerously interfering, i.e., ventricular systole begins while auricular systole is still in progress. The auricular valves must therefore be open. But in interference waves of type II, period (*U* to *V*) in which auricular and ventricular systole have the same relation and offer similar opportunity for regurgitation, the final intraventricular tension does not drop, but shows a rise, (*V* to *W*) as sudden as the fall (*C* to *A*) in type I. If regurgitation does occur it does not keep pace with the enhancing effects of filling due to the partially stoppered auricular systoles. In no case does the intraventricular tension during the periods in which disturbed valvular action could occur fall below that maintained by venous pressure alone (period *A* to *B*, and *U* to *V*). The oscillations in output and tension must therefore be explained by factors other than disturbed valvular action, i.e., to the enhancing effects secondary to the increased ventricular filling.

Filling Effect of Auricular Systole. If valvular action is neither dependent on, nor disturbed by auricular systole, the effects of auricular systole must be due to increased ventricular filling. Myocardiograph records show the filling effect to be considerable. (see lower tracing fig. 11 *X E* to *F*). This is a myocardiograph tracing of the ventricle with the heart in complete block. The ventricles are initiating their own rhythm of approximately one ventricular to three auricular cycles. The first sudden increase of ventricular volume is due to the filling action of venous pressure or to auricular systole, depending upon whether or not auricular contraction is in progress at the onset of ventricular relaxation. The venous pressure in this instance was 4.5 cm. of blood. The succeeding steplike increases are due in each case to the filling action of auricular systole. Note the relative importance of the two filling forces and the permanence of the filling due to auricular systole.

The filling effect is brought out still better in the myocardiograph tracing of figure 11 (*Y*) in which the auricles are inhibited by vagus stimulation, one escaped auricular contraction occurring. The only filling force is venous pressure, with the exception of one ventricular cycle, in which venous pressure and auricular systole both are effective. The venous pressure is 5 cm. of blood, the ventricular rate 72 per minute. Keeping in mind the surface-volume relation it would appear that the degree of filling due to auricular systole is even greater than that due to venous pressure. The record is of particular value in that the venous pressure is relatively high and the ventricular rate slow —offering ample opportunity for filling by venous pressure.

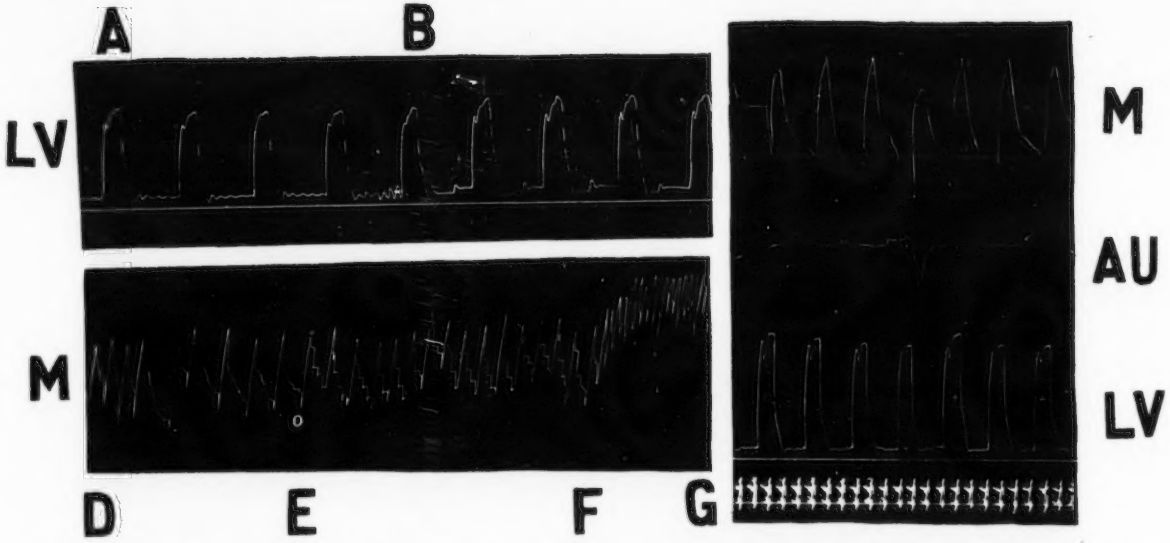

Fig. 11 (*X*). *L.V.*, Left intraventicular tension curve showing oscillations of tension due to auricular fibrillary contractions and to auricular systole. *M.*, Myocardiograph tracing showing volume length changes due to fibrillary (*D-E*) and normal auricular contraction (*E-F*). *F.G.*, ventricles stimulated at a more rapid rate.

Fig. 11 (*Y*). Escape auricular systole during vagus inhibition showing effect on ventricular volume and resulting tension curve. *M.*, Myocardiograph tracing. *AU*, auricular left contraction; *LV.*, intraventricular tension.

Other points of interest in connection with this record are the increased duration of contraction, the increased final tension and the failure of the ventricle to reach its preceding final volume—all as a result of the escaped auricular contraction.

Though the output of this ventricular systole is considerably increased, the final volume is greater than that of the preceding ventricular systoles. With this in mind, changes in ventricular volume other than those occurring with each auricular systole may be considered in connection with interference waves (see myocardiograph tracing of figure 7). In addition to the sudden volume increase accompanying auricular systole more gradual oscillations of both initial and final volume occur rhythmically with each interference wave. At (*A*), (*C*), and (*F*) auricular systoles are stoppered and the ventricles filled by venous pressure alone. At (*B*), (*D*), and (*E*) the auricles are free to inject additional blood into the ventricles. While the effect of auricular systole is annulled, initial and final volume are at their minimum. Where auricular systole has maximum efficiency the initial and final volumes are largest. The increase of initial and final volume from (*A*) to (*B*) is due to the increasing effectiveness of auricular systole and to the increasing failure of the ventricles to empty themselves completely i.e., the auricular systole furnishes the ventricles with more blood than they can handle. Accumulation of blood in the ventricles therefore occurs. The record shows that the greater the initial volume the more poorly do the ventricles empty themselves, and vice versa, the smaller the initial volume the more completely do they empty. But even at (*C*) where the ventricles were moderately filled they fail to empty themselves completely. Such records show that ventricular volume per se is not the factor determining ventricular efficiency; but that the secondary factors accompanying the volume changes determine this primarily.

Though the volume output increases with increasing volume, the enhancing effects of the secondary factors are not sufficient to maintain the final volume obtaining during poorer ventricular filling.

ANALYSIS OF ENHANCING FACTORS ACCOMPANYING INCREASED VENTRICULAR VOLUME

The slow oscillations of volume, noted in fig. 7, resemble the volume changes described by Patterson, Piper and Starling,[11] but have a

[11] Patterson, Piper and Starling: Journ. Physiol., 1914, xlviii, 465.

somewhat different origin. These observers find that any condition which increases the demands on the heart, whether it be increased capillary resistance or increased venous pressure, produces increased ventricular volume which in turn is accompanied by increased ventricular efficiency. In view of the relation of length of muscle fiber to strength of contraction they see in this reaction a regulative mechanism by which the blood accumulates in the heart until the length of ventricular fiber (strength of contraction) is great enough to meet the new demands. To quote from them:

> We thus find no constant connection between the diastolic tension and the succeeding contraction, though as a rule these two quantities will be altered together. But we do find a direct proportion between the diastolic volume of the heart (i.e., the length of its muscle fibers) and the energy set free in the following systole.

Further

> We see from these tracings that an invariable condition of increased contractile stress is increased initial length of muscle fiber. This may be accompanied or brought about by increase in the initial tension of the muscle fiber, but the two conditions are not invariably connected, and we shall find later other cases in which length varying without changes in tension has brought about its proper effect on the strength of contraction of muscle.

From the above it is obvious that these investigators consider length of ventricular muscle as the factor of primary importance. They do not look upon initial intraventricular tension as exerting an influence on ventricular contraction.

The contemporaneous work of Straub[12] lays stress upon another factor. Employing the same methods as Patterson, Piper and Starling, and making some of the same fundamental observations Straub arrives at entirely different conclusions. Finding ventricular diastolic tension, as well as ventricular diastolic volume to increase with increased demands on the heart, he attributes the accompanying increased ventricular efficiency to increased initial tension. In other words he considers variation of initial tension as the regulative mechanism of cardiac efficiency. He states:

> Wie die Druckkurve ausweist, bedeutet bei unseren Versuchsbedingungen die vermehrte Anfangsfüllung eine vermehrte Anfangsspannung, d. h. der diastoliche Minimaldruck ist gestiegen. In diesen Vermehrungen der Anfangsspannung

[12] Straub: Deutsch. Arch. f. klin. Med. 1914, cxv, 531.

liegt nun der Grund, der die anpassung des Herzmuskels an die erhöhte Überlastung ermöglicht. Mit erhöhte Anfangsspannung wird nach den Zuckungsgesetzen des Skelettmuskels und des Froschherzventrikels das Druckmaximum erhöht, d. h. der Ventrikel leistet sofort erhöhte Arbeit und ist nunmehr imstande das ganze Schlagvolume gegen den vermehrten Wiederstand auszuwerfen.

From these quotations it is obvious that the views concerning the relative effects of initial length and initial tension of muscle fiber on contraction are still divided, and each factor has been considered as a means of regulating cardiac efficiency. It therefore seemed to the point to determine if possible the relative importance of these factors in contraction of cardiac muscle.

If the load of striated muscle is increased the work performed increases in a definite fashion. If the muscle is afterloaded initial length and initial tension remain constant; if not afterloaded, initial length and initial tension increase with each increase of load. The greater efficiency per given load in the second case has been attributed with equal emphasis to increased initial length and to increased initial tension. But in such experiments the two factors vary together. The difficulty of determining the relative importance of each is evident.

The properties of the auricular muscle of the turtle permit independent variation of initial length and initial tension and therefore offers an opportunity of separate analysis of these two factors, in simple experiments with conditions under easy control. In such experiments we may keep the actual as well as the filling tension constant while the length of fiber changes, and we find increased strength of contraction to accompany either increased length of fiber while initial tension remains constant or increased tension while initial length remains constant.

If these results obtained on the turtle's auricle can be applied to the mammalian heart, the work of Patterson, Piper and Starling and of Straub require broader interpretation. It would seem that under conditions in which initial intraventricular tension is low and varies but little, the factor of initial length of fiber is by far the more important of the two, but with high initial tension the factor of tension may grow in importance. But the interpretation of cardiodynamics cannot be limited to the factors of initial length and tension of the muscle fiber for another factor comes simultaneously into play. This factor is the surface-volume relation accompanying volume changes of the ventricle. The volume of a growing sphere increases more rapidly than the surface. If we consider the ventricle as roughly spherical—

and its walls the surface—its contents the volume, the importance of this surface-volume relation is evident. It means that the greater the ventricular volume the greater the output per unit length of muscle shortening.

At least three factors are important in regulating the efficiency of the heart. When the demands on the heart are increased, ventricular volume increases until the enhancing factors of initial length of fiber, initial tension of fiber and the surface—volume relation meet the new demands.

(A) Length of Fiber. Length of fiber is the important factor determining the liberation of contractile energy. In the present experiments the final intraventricular tension was used as the gauge to strength of contraction. Since the tension developed depends on the amount of blood forced through the given resistance per unit of time, the tension developed may not depend entirely upon the amount of energy liberated but also upon the manner in which it is utilized, for instance the amount of muscle shortening and the effectiveness of the given shortening which occurs with various initial volumes. Strength of contraction or tension developed may therefore be a factor of "initial volume-length" rather than initial length alone. This term, "initial volume-length" will therefore be used to designate these factors; but where the factors of volume or length are specifically in question they alone will be mentioned.

For simplicity the factor of initial length will be arbitrarily considered alone in two given cases.

In the course of many interference waves produced in these experiments, initial right intraventricular tension varied but little as a result of varying effectiveness of right auricular systole; but the usual oscillations of initial length of ventricular fiber occurred. In such cases the final tension developed varied directly as the initial length of fiber, agreeing with the results obtained on turtle's auricular muscle (see tracing *R.V.*, fig. 7).

The same relation of final tension to initial length of fiber is also displayed by the left ventricle. During certain periods of the interference waves the left intraventricular initial tension may remain constant for a number of cycles though a decrease of initial length of fiber occurs. (See fig. 16). In such instances too, the final tension varies directly as the initial length of fiber. But, as will appear later, initial length of fiber is not the only factor determining the strength of a given contraction. The factors of initial volume, and surface-volume

relation accompanying volume changes may influence the energy liberated in a given contraction, the effectiveness of the liberated energy and also the effectiveness of muscle shortening. It is well, therefore, to look upon the changes of final tension as a volume length effect rather than length alone.

Experiments on the auricle of the turtle showed duration of any given contractile tension as well as magnitude of final tension to be increased by increased initial length of fiber. With the conditions obtaining in the present experiments increasing initial volume-length increased duration as well as strength of contraction (see fig. 12 which shows left intraventricular tension curves only). Curves (*A*, 1 and 2) were taken from an interference wave with volume-length at its maximum and minimum respectively, that is, when auricular systole was at maximum and minimum efficiency. In this particular instance the increased duration of contraction is relatively greater than the corresponding increased magnitude of tension, a factor of no little significance. This increased duration of contraction comes out even more clearly in curves (B 1 and 2 fig. 12), in which there is only one auricular to two ventricular cycles. For ventricular cycle (*B*, 1, fig. 12) the ventricle is filled by venous pressure and auricular systole for (*B*, 2) by venous pressure alone. These cycles (*B*, 1 and 2) correspond approximately to ventricular cycles (*A*, 10 and 11, fig. 6). The difference in duration of contraction in the case of cycles (*B*, 3 and 4, fig. 12) is not so great. These cycles correspond approximately to cycles (*A*, 8 and 9, fig. 6), which explains the difference.

The practical significance of increased duration of contraction as well as strength of contraction under physiological and pathological conditions need scarcely be pointed out in this paper.

The relation of strength and duration of contraction as affected by volume length will be further elucidated in the section on surface-volume relation.

(B) Initial Tension. In this work, in no instance was initial tension the only varying factor, and no definite data concerning the effect of initial tension on ventricular contraction was obtainable. The significance of initial tension in relation to cardiodynamics must necessarily be a matter of inference. But if the results obtained on the turtle's auricle can be applied to the mammalian heart it is evident that initial tension might at times be an enhancing factor of some importance. In the course of an interference wave the initial left intraventricular tension varied from approximately zero to 20 mm. Hg, see figure 15.

The increase of initial tension is due in part to the increased output of the right heart and in part to increasing effectiveness of left auricular systole. Whatever the cause, it means that this tension is stored in the stretched ventricular muscle during diastole as potential energy. When the muscle contracts this potential energy is liberated as dynamic energy and is effective in assisting the active contraction in expelling the blood. In addition to this mechanical factor we must bear in mind a possible enhancing effect of initial tension on the processes of muscular contraction.

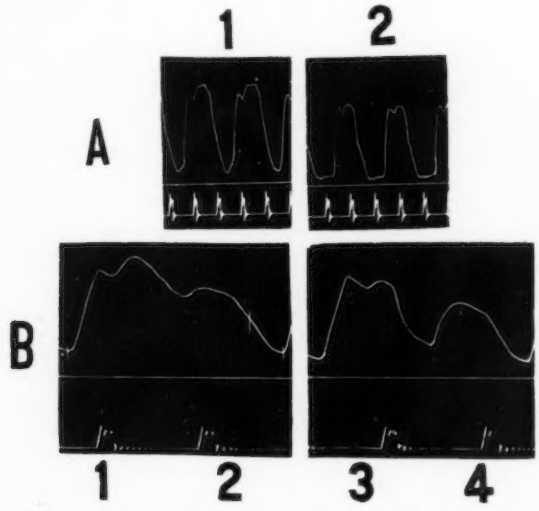

Fig. 12. Effect of auricular systole on duration and strength of ventricular contraction. *A* and *B*, left intraventricular tension curves from interference waves of type *B* and *A*, figure 6 respectively.

Patterson, Piper and Starling suggest that increased initial tension may put the muscle on a slight stretch taking up ventricular slack and thereby minimizing waste contraction. This factor is probably of more significance in the flaccid right ventricle than in the left ventricle. A further suggestion along these lines might be offered. The ventricle tends to assume a more spherical shape with increased intraventricular tension, this change occurring before the ventricle decreases in volume. If this spherical shape could be produced by increased initial tension (auricular systole or venous pressure) waste contraction would be

decreased still more, i.e., the very first part of contraction would be effective in expelling blood, and the rate of shortening of muscle during the first part of contraction would be decreased.

It would be of interest to know the optimum initial tension for cardiac efficiency. This undoubtedly depends on a number of factors, the elasticity of the muscle, the relative economy with which the muscle sustains a high constant venous pressure and the rapid short-lived tension due to auricular systole, and the enhancing effects of various tensions on active contraction itself. It has been suggested that the sarcoplasm may under certain conditions bear the constant tension obtaining between the clonic contractions. Other suggestions are found in the literature pointing to a difference in the metabolism of sarcoplasm and of the fibrillae of muscle, one being a protein metabolism, the other a carbohydrate metabolism. It is likewise stated that resistance of smooth muscle (which possible has properties similar to sarcoplasm) to constant tension is very economic. If the above is true it is plausible that even high constant initial tension resulting from venous pressure might have an enhancing effect on muscular contraction. But the short duration of increased initial tension produced by auricular systole may be of particular value.

(C) Surface-Volume Relation. The relation of surface-volume to cardiodynamics may be considered from three points of view.

1. Influence on the effectiveness of a given contraction (muscle shortening).

2. Influence on the amount of contractile energy liberated in a given contraction.

3. Influence on the effectiveness with which the given liberated energy in utilized.

1. The influence of surface volume relation on the effectiveness of a given contraction will be considered first, for it will at the same time illustrate what is meant by that relation. The volume of a sphere varies as the cube of the radius, and the surface area of a sphere as the square of the radius. It therefore follows that the volume of a growing sphere increases more rapidly than the surface. This relation is shown in Table II for spheres of different radii. The significance of this relation, when the ventricular walls are considered as the surface and the contents as the volume, was pointed out before, and is illustrated by quantitative data collected in Table II last column:—the decrease of volume per unit decrease of surface area. The circumference-area relation of circles is similar to the surface-volume relation of spheres. It

is therefore simpler to use circles, representing sections through the ventricles, to show diagramatically the significance of this surface-volume relation (see fig. 13).

In one instance (*A*, fig. 13) the ventricle is filled to a radius of let us say 3 cm., in the other to 10 cm. Granting in case (*A*) that the ventricular fiber (ventricular ring) shortens one-third its initial length or 6 cm. it contracts to a circle with a "volume" of 12 cm. The output is 16 cm. The same contraction of 6 cm. in case (*B*) with a radius of 10 cm. produces an output of 60 cm. But in this instance, 6 cm. is only one-tenth of the circumference. We know that a long muscle fiber contracts much more than a short muscle fiber and should the ring contract one-third its length as in case (*C*) the volume output would be 178 cm.

The application of this relation appears in the myocardiograph tracing of figure 7. At (*C*) and (*F*) where initial volume is smallest, the magnitude of contraction is small, and ventricular output is consequently

TABLE II

RADIUS	SURFACE AREA	VOLUME	VOLUME CHANGE PER UNIT CHANGE OF SURFACE
cm.	*sq. cm.*	*cc.*	
3	108	108	1.0
6	432	864	2.0
10	1200	4000	3.3
14	2352	10976	4.6
20	5024	33158	6.6

small; at (*E*) and (*G*) where initial volume is greatest, magnitude of contraction is also greatest and the output therefore is markedly increased.

An increase in volume corresponding with radii of 3 and 10 cm. would be extreme in normal hearts, but such an increase in association with the change from a normal to a pathological condition is met with and illustrates how adaptive the mechanism of dilatation is, especially when considered along with the increasing strength of contraction accompanying volume increase.

Under certain experimental conditions the volume of the ventricles in some instances probably doubled in the course of an interference wave. It is of interest to analyze the effect of surface-volume relation alone associated with such a volume change.

Taking a ventricular circle with a radius of 3 cm., a circumference of 18 cm., and a "volume" of 27 cm. the output per unit shortening

of muscle is 1.5 cm. Doubling the "volume" produces a circle with a radius of 4.1 cm., a circumference of 26 cm., and a "volume" of 54 cm. With this larger initial volume the output per unit shortening of muscle is 2 cm. an increase of 33 per cent. But if the ring contracts in proportion to its initial length, the output is increased 60 per cent. An increase of this magnitude is commonly noted in the course of an interference wave.

Making the same assumptions in regard to ventricular relaxation as to ventricular contraction, surface-volume relation should enhance ventricular filling by venous pressure in the same way as it increases the effectiveness of a given contraction of muscle during systole.

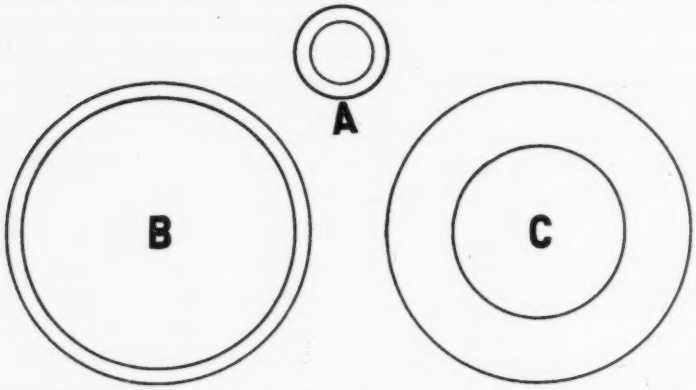

Fig. 13. Relation of surface volume to effectiveness of given ventricular muscle shortening. In *A* and *B* the contraction of the ring is equal with an output of 16 and 60 cm. repectively. In *C* contraction is proportional to that in *A*. The output is 16 and 178 cm. respectively.

Since auricular systole increases both initial and final ventricular volume it increases ventricular volume in two ways: (a) Directly—by injection of blood into the ventricles; (b) Indirectly—by supplying the ventricles with more blood than they can handle. Final ventricular volume is thereby increased and this increased final volume in itself increases fillng by venous pressure.

With the same shortening of ventricular musculature in two different initial volumes the relation of surface volume to effectiveness of contraction is obvious. This relation is important in utilizing the increased energy resulting from increased length of ventricular fiber. For example

in some hearts when cardiac demands are increased by i creased filling initial volume alone is markedly changed, final volume may remain more nearly constant. That is the ventricle empties itself almost as well when initial volume is large as when it is small. Such instances are indicative of a rapid increase in strength of contraction as a result of increased length of fiber. If there were no means of utilizing this strength the heart would be emptied before contraction was completed. The surface-volume relation prevents this. Since the efficiency of these ventricles varied approximately as the ventricular volume, it points to the nice adjustment of strength of contraction and ventricular volume which occurs in some hearts when in good condition.

2. Influence of surface volume relation on the amount of contractile energy liberated in a given contraction. The discussion of this influence is based upon the work of Blix[13] and Hill.[14] Blix is of the opinion that the amount of contractile energy liberated in muscular contraction is a function of the exposed area of certain chemically active surfaces within the fibrillae at the time of excitation. This would make initial length of fiber the strength determining factor. Hill, however, believes that the processes at the chemically active surfaces producing the contractile energy, require an appreciable time for their completion, therefore the length of muscle during the early part of contraction as well as the initial length, determines the contractile energy liberated in any given contraction. It is in this connection that ventricular volume and surface-volume relation come into play in influencing the contractile energy liberated. The influence of initial length alone was discussed before on page 291.

As was pointed out by Patterson, Piper and Starling increased initial volume by increasing final volume would in itself insure increased length of fiber throughout contraction thereby increasing strength of contraction. This is a manifestation of surface-volume rather than of volume alone and therefore becomes of increasing importance the more poorly the ventricle empties itself. But in many instances in which the ventricular muscle is in good condition, and the demands on the heart are increased, final volume, as indicated by the myocardiograph tracing, does not increase nearly as rapidly as initial volume, i.e., the ventricle empties itself almost as well when initial volume is large as when it is small. This means that the secondary enhancing factors have been increased by increased initial volume sufficiently to handle

[13] Blix: Skand Arch. f. Physiol., 1902, xii, 52.
[14] Hill: Journ. Physiol., 1913, xlvi, 434.

almost perfectly the blood received by the ventricles. In such instances surface-volume relation rather than the volume itself (final volume) is the factor of importance increasing both strength and duration of contraction, for the greater the ventricular volume the greater the output per unit shortening of muscle. But with a given contractile stress there is a limit to the output per unit of time and unless this stress is disproportionately increased by increased length of ventricular fiber, the tendency of the increased volume, though the muscle may contract to its minimum, will be to make the early part of contraction isometric and in that way increase the strength and duration of contraction.

3. Influence of surface volume relation on the effectiveness with which a given available energy is utilized. In the contraction of any strip of muscle we look upon the liberation of contractile energy and the resulting contractile stress as running a parallel course. That is stress is directly proportional to the liberated contractile energy. This is not the case in hollow spheroid contractile organs like the heart and was taken into account by Stephen Hales, 1733,[15] in determining the strength of ventricular contraction. In the case of a muscle strip, we have a linear pull and no changing muscle surface to consider. In the heart the muscle as a surface must support the developed tension. It is therefore possible in the course of systole for the contractile stress to increase though the contractile energy is decreasing. This would be dependent on the relative rates, with which contractile energy and the surface over which this energy is spread decrease.

The form of the tension curve produced by normal ventricular systole must therefore be dependent upon these two factors and on the amount and nature of the peripheral resistance.[16]

Assuming the ventricle to be spherical, and computing the tension developed under isometric conditions for different initial volumes, but with the liberation of equal amounts of contractile energy, we obtain data of considerable interest in relation to the importance of the internal surface obtaining with different volumes. In Tables III (*A*) and (*B*)

[15] Stephen Hales: Statical Essays, 1733.

[16] As this paper goes to press, I find that Patterson and Starling in a footnote previously overlooked (Journ. Phys., 1914, 48, 358) make a similar suggestion concerning the form of the tension curve and also point to the mechanical advantage of systole resulting from decreased surface. In the present paper, this factor is discused quantitatively as far as it can be, and is considered in connection with its varying importance with different initial volumes.

contractile energy sufficient to produce a tension of 100 mm. Hg. when the ventricle has a radius of 3.1 cm. is used in the computation. In Table III (*A*) the intraventricular surface, the volume, and the stress

TABLE III-A

R.	VOLUME	SURFACE	STRESS
	cc.	*sq. cm.*	*mm. Hg.*
1.0	4.19	12.6	952.5
1.3	9.21	20.9	570.1
1.6	17.13	32.2	372.7
1.9	28.70	45.3	264.4
2.2	44.62	60.8	197.3
2.5	65.36	78.5	152.9
2.8	91.97	98.5	121.5
3.1	124.86	120.0	100.0
3.4	164.66	145.1	83.0
3.7	212.22	171.9	69.8
4.0	268.16	200.9	59.9
4.3	333.10	232.2	51.7
4.6	407.91	265.0	45.2
4.9	492.95	301.5	39.8

TABLE III-B

CONTRACTION FROM $R^1 - R^2$	VOLUME OUTPUT	PER CENT INCREASE OF TENSION. END OF SYSTOLE (R^2) COMPARED WITH BEGINNING OF SYSTOLE (R^1)	PER CENT INCREASE OF TENSION, IF OUTPUT WERE 50 CC. IN EACH CASE
R^1 R^2	*cc.*		
4.9 – 4.0	224.8	50.5	11.2
4.6 – 3.7	195.7	54.4	13.9
4.3 – 3.4	168.4	60.5	18.0
4.0 – 3.1	142.3	66.9	23.5
3.7 – 2.8	120.3	74.0	30.8
3.4 – 2.5	99.2	84.2	42.4
3.1 – 2.2	80.2	97.3	60.7
2.8 – 1.9	63.2	118.6	93.8
2.5 – 1.6	48.3	143.8	148.8
2.2 – 1.3	35.4	189.0	267.0
1.9 – 1.0	24.5	298.0	607.9

developed in ventricles with different radii are given. This relation of stress to the radius, that is, the effectiveness of a given energy to develop tension with varying size of the ventricle is plotted in figure 14, *X*. This figure shows the varying importance of the surface-volume re-

lation with different initial volumes—for the efficiency of the contractile energy increases more rapidly per unit of muscle shortening, the smaller the initial volume, a factor which would point to an optimum initial volume for maximum utilization of contractile energy. The significance of this point is brought out in Table III (*B*). Column (1) represents ventricular contractions from initial radius (R') to final radius (R^2). These contractions are all of equal magnitude. Column (2) represents the output resulting from these contractions; column (3)

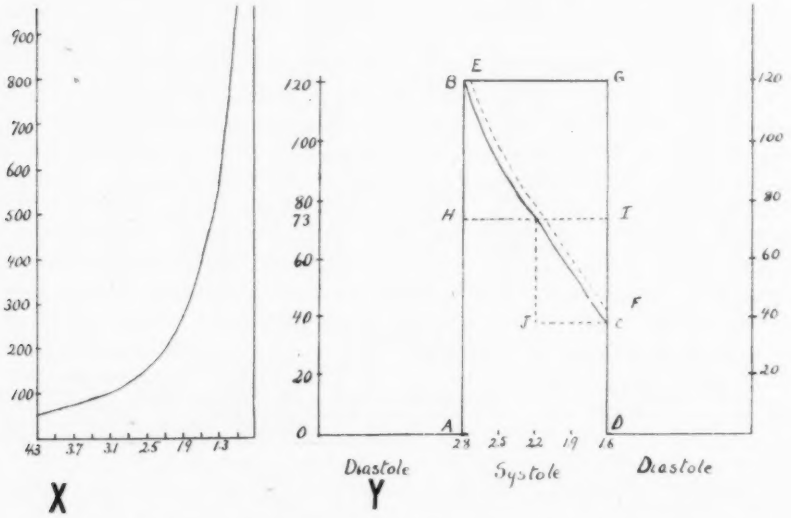

Fig. 14. *X*, Curve of contractile stress for a constant amount of contractile energy liberated in spheres with different radii. Ordinates—stress in mm. Hg. Abscissae—radius of spheres. *Y*, Hypothetical tension curve with a given curve of liberated contractile energy and a parallel curve of decreasing intraventricular surface. (For further explanation see text.)

represents the resulting increased efficiency of the contractile energy, i.e., the tension developed with the final radius as compared with that developed with the initial radius. Note that the effectiveness with which the liberated energy is utilized increases rapidly as the initial volume decreases, even despite the decreasing output occurring with the equal muscle shortening. But assuming a constant and approximately normal systolic discharge of 50 cc. the increasing effectiveness of

the contractile energy is very much greater (see Column 4). With such a discharge the increased effectiveness of the energy is only 11 per cent when the initial radius is 4.9 cm. and 607 per cent when the initial radius is 1.9 cm.

Normal ventricular volume relations in the ventricular cycle are approximated in the contraction from a radius of 2.5 cm. to 1.6 cm. The output is 48 cc. and the residual volume 17 cc. In such an instance the resulting decreasing surface would produce increased efficiency of the energy for development of tension of approximately 148 per cent.

But the available contractile energy in muscular contraction does not remain constant throughout systole as assumed in these hypothetical cases. Consequently the ability of the ventricles to develop tension will depend largely upon the nature of the processes of contraction, particularly upon the rate at which the contractile energy is liberated or stored as potential energy and the rate at which this energy is dissipated.

Upon muscular excitation, according to Hill[17] certain processes occur at the chemically active surfaces which produces a change in the elasticity of the muscle thereby storing potential energy which is at the disposal of the muscle to perform work or develop heat. According to Hill, this new state of elasticity is of short duration. If the muscle is given an opportunity to contract during that period, work is performed, if not, the potential energy is dissipated as heat. At excitation therefore potential energy is stored just as when muscle is actively stretched. Applying this conception to decreasing intraventricular surface during ventricular systole we see a compensating mechanism between decreasing intraventricular surface and decreasing available energy. Assuming the muscle to be elastic, comparable to rubber tissue, in the so called stretched condition at the onset of contraction, the potential or available contractile energy is at its maximum. As the muscle shortens the contractile energy diminishes but counteracting this, is the decreasing surface which increases the efficiency of the remaining contractile energy. If in the course of muscle shortening the contractile energy is dissipated at the same rate as the intraventricular surface decreases, the tension curve would have a horizontal plateau. Such conditions are plotted in figure 14 (*Y*). Curve (*ABCD*) represents the liberation and dissipation of contractile energy in the course of systole, (*AB*) the sudden liberation, (*BC*) the dissipation during shortening and

[17] Loc cit.

(*CD*) the sudden cessation of the new elastic state of the muscle. (*EF*) represents the decreasing intraventricular surface, (*ABGD*) the resulting tension curve.

The significance of this relation is apparent. It works toward complete utilization of liberated energy and minimizes waste residual contraction. For example if the peripheral resistance during systole is represented by (*HI*) the entire systole is effective in expelling blood. If the decreasing surface did not accompany decreasing available energy there would be a waste residual contraction (*JC*) amounting to fifty per cent of the entire contraction. It seems that this compensating mechanism between decreasing intraventricular surface and decreasing strength of contraction must play an important part in cardiodynamics, whether Hill's conception of muscular contraction holds or not. A breaking down of this mechanism would result in residual waste contraction. For instance, should the contractile energy be dissipated faster than the surface decreases, a tension lower than the peripheral resistance might result. Under such conditions there would occur prolonged contraction at the end of systole with the development of considerable tension below peripheral resistance but with no output. Such maintenance of tension without output does occur as shown by Patterson, Piper and Starling and others, and it would seem that its explaination consists in the failure of the surface effect to compensate the decreasing energy.

In regard to the question of the most efficient relation of available contractile energy to the supporting surface it is of interest to analyze the nature of ventricular contraction. Ventricular systole meets with two resistances: capillary resistance and stretching of the arterial walls. Capillary resistance remains constant. Since the resistance to stretching increases with filling, the ventricular contraction resembles auxotonic contractions in which resistance to shortening of the muscle increases as contraction proceeds. The major work of the heart is the storage of potential energy in the arterial walls. In this connection it would seem that the decreasing surface tending to increase or maintain the strength of contraction as systole proceeds and resistance increases is particularly valuable.

As a principle in cardiodynamics the decreasing surface must be important; but as a factor in the "adaptive volume reaction," that is the increased initial volume occurring whenever cardiac demands are increased, the relation is not so clear. From Table III (*B*), however, it would appear that the disadvantage to the ventricle as far as economic

utilization of energy is concerned is considerably increased with increase of initial volume; for with the same given output the increased efficiency of a given constant energy, is approximately 607 per cent with a radius of 1.9 cm. and only 11 per cent with a radius of 4.9 cm. This question however needs amplification. The effect of initial volume on utilization of energy depends upon our conception of the nature of muscular contraction. If Hill's explanation is accepted, the disadvantage of increased volume is not as great as appears in the Table III (*B*). For the available contractile energy during any period of contraction would depend largely upon the amount of muscle shortening. Since the shortening with large initial volume is less per given output, the compensating mechanism of decreasing surface is not so essential. But the available contractile energy in muscle is not merely a matter of shortening; it is also a factor of time, for the contractile processes last a short time only. They develop and disappear in a definite fashion. In isometric contractions, tension rises suddenly, is maintained for a short time at a varying level, and then falls, but at a much slower rate than the rise. This fall of tension in isometric contraction is a factor of time, for the muscle does not shorten and this decreasing available energy as affected by time would in all cases be more economically utilized when initial volume is small than when initial volume is large.

The relation of surface to tension leads indirectly to the question of the limit of the adaptive volume reaction in response to increased cardiac demands. Most of the factors involved have been analyzed. It is seen that these factors vary in relative importance and in different proportions under varying conditions; that they are inseparably interrelated and affect each other in different ways:

Increased initial length and increased initial tension increase the strength of contraction. Increased initial volume by maintaining a greater length of fiber throughout contraction likewise increases the contractile energy liberated. The manner in which surface-volume relation increases the duration of contraction and the energy liberated, and the effectiveness of a given shortening of muscle have been discussed. Most of the factors mentioned are the result of increased ventricular filling and tend to increase strength of contraction. But accompanying increase in strength of contraction there are certain antagonising factors which eventually lead to the breaking down of the adaptive volume reaction. From Table III (*A*) it would appear that chief among these factors is the increasing intraventricular surface

accompanying volume increase.[18] In addition the relatively smaller decrease of surface per unit output with larger initial volumes may lead to less economic use of the expended energy than occurs with smaller initial volume and in turn lead to collapse. Whether the high constant initial tension obtaining in extreme cases exerts a deleterious or enhancing effect on cardiac efficiency is difficult to state.

In some hearts all the factors mentioned work so smoothly that the two primary factors of energy and effectiveness of the given energy are well coordinated. With the ventricular muscle in good condition increase of initial volume from increased demands may lead to a much smaller increase of final volume, i.e., the efficiency of the ventricles varies approximately as their volume giving the impression that volume per se is the factor determining ventricular output, when in fact, the factors secondary to increased initial volume are working so perfectly that they are not obvious. But if these hearts are exhausted or the demands increased, the working of the secondary factors becomes more apparent. If the increased strain on the heart becomes too great the adaptive volume reaction suddenly breaks down. The reason is obvious. The effect of increasing strength of contraction is increasingly counteracted by the factors mentioned. When these two antagonising factors equalize each other the heart is in a precarious condition. Any additional strain would produce collapse.

Another point of interest is a detail in the method by which the ventricles meet increased demands. The demands on the ventricles may be increased in two ways: (1) by increasing the peripheral resistance; or (2) by increasing ventricular filling. The first method was not studied in this group of experiments. But Patterson, Piper and Starling, and Markwalder and Starling[19] find that when the peripheral resistance is raised, ventricular volume increases until the output is equal to that obtaining at the original resistance. Since heart rate is constant it follows that with the larger initial volume the actual contraction of the ventricular fibers is less though the energy liberated in each contraction is increased. But if the demands on the heart are increased by increasing ventricular filling, e.g. progressively increasing the effectiveness of auricular systole in an interference wave, the initial volume increases, but in this case the volume output and magnitude of contraction likewise increase, see figure 7.

[18] Patterson and Starling: Journ. Physiol., 1914, xlviii, 357.
[19] Markwalder and Starling: Journ. Physiol., 1914, xlviii, 348.

RELATIVE IMPORTANCE OF VENOUS PRESSURE AND AURICULAR SYSTOLE

Venous pressure and auricular systole are the two important forces producing ventricular filling. Since interference waves give the output maintained by venous pressure alone and by combined venous pressure and auricular systole, they offer an opportunity for determining the relative importance of each with varying magnitudes of venous pressure. Table I gives some of the result obtained. Henderson states that auricular systole is important only when very low venous pressures exist. According to him, auricular systole has no filling effect when the venous pressure is above the "critical" pressure of 5 cm. of blood.[20] But it will be noticed that even with a venous pressure of 9 cm. of blood auricular systole increased ventricular output 35 per cent over that maintained by venous pressure alone (see fig. 17); and in other experiments, in which output was not measured, marked oscillations of blood pressure occurred in the course of interference waves though venous pressure was as high as 15 cm. of blood. It might be expected however, that the importance of auricular systole would fall off markedly with increasing venous pressure. But this does not necessarily follow, for auricular systole is an additional force to venous filling and any additional force should add its filling effect, thereby increasing ventricular efficiency. In addition the effectiveness of auricular contraction is increased by increased venous pressure in every respect just as is ventricular contraction increased by increased ventricular filling. As long as increased ventricular volume increases ventricular efficiency,—auricular systole should be effective in increasing this efficiency regardless of the venous pressure obtaining. No definite statement can be made with regard to the relative importance of venous pressure and auricular systole. It depends on many variable factors,—strength of auricular systole, duration of ventricular diastole, rate of ventricular relaxation, and resistance which ventricular muscle offers to stretching. This is brought out in tracings (16) and (62) Table I taken from the same animal with same venous pressure. In one case ventricular rate is 138 in the other 298 per minute. In the latter auricular systole is relatively more important than in the former. This probably is due to the shortened period of diastole, and to the fact that the venous pressure was not sufficient to produce ventricular stretching, while auricular systole was.

[20] Henderson and Barringer: This journal, 1909, xxxi, 352.

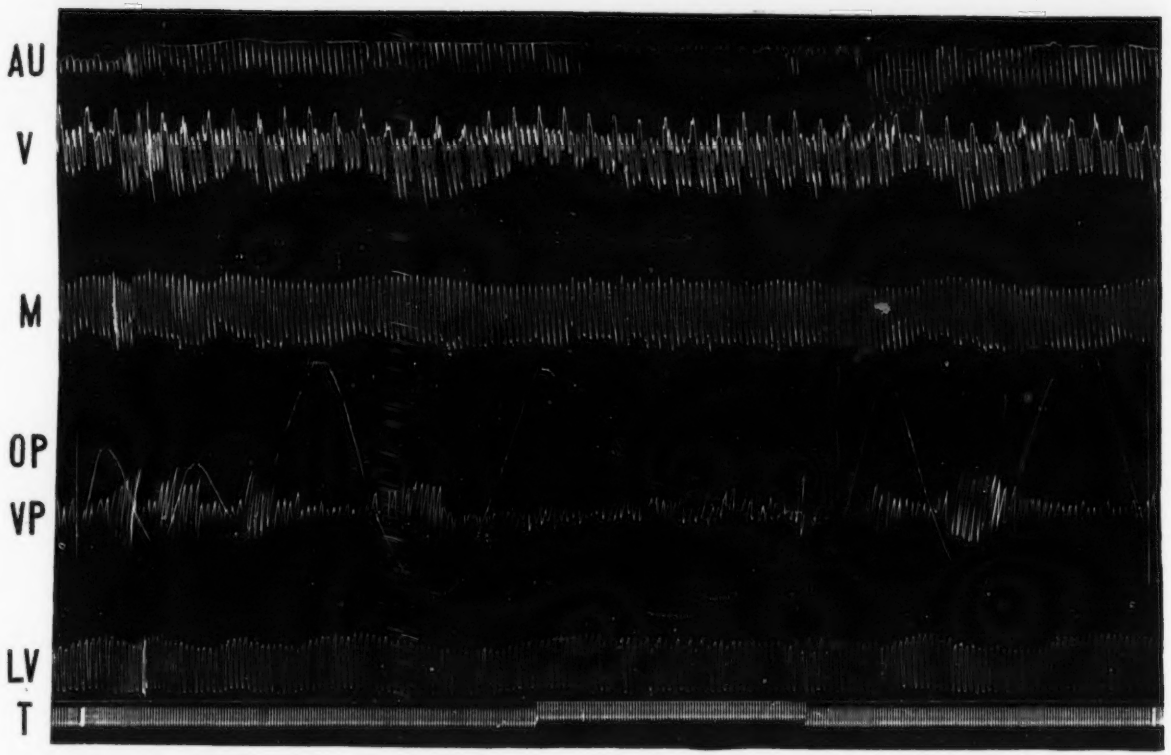

Fig. 15. Effect of faradic stimulation of auricles at the crest of an interference wave. *AU.*, auricles; *V.*, ventricle; *M.*, myocardiograph; *O.P.*, left ventricular output; *V.P.*, venous pulse; *L.V.*, left intraventricular pressure; *T.*, time in seconds.

INFLUENCE OF SO-CALLED AURICULAR FIBRILLARY CONTRACTIONS ON VENTRICULAR EFFICIENCY

The so-called fibrillary contractions of the auricles produced by faradic stimulation are usually considered as having little filling effect upon the ventricles. This attitude may largely be accounted for by the association of these contractions with fibrillary contractions as seen in the ventricles which as is well known have practically no efficiency.

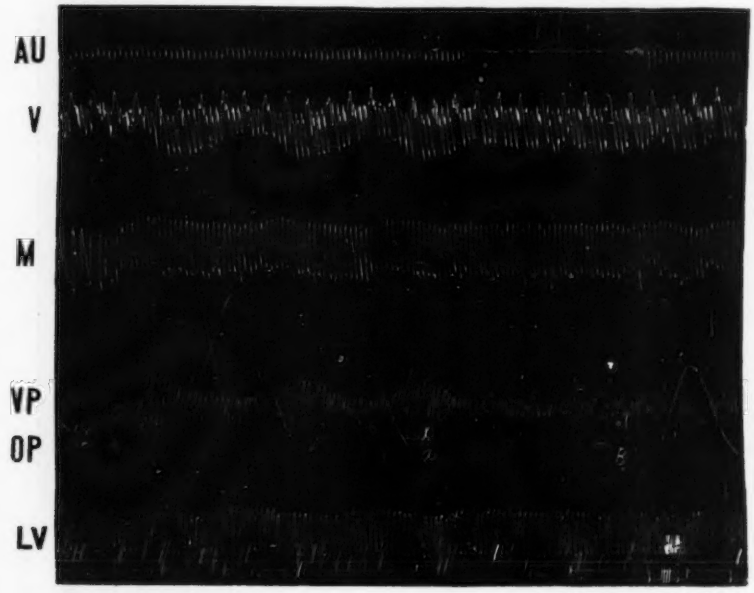

Fig. 16. Effect of faradic stimulation of the auricles at the trough of an interference wave. Stimulation at *A*; recovery at *B*.

It is pertinent to discuss briefly the nature of auricular contractions as obtained by faradic stimulation of the auricles.

Two types of auricular contraction resulting from such stimulation, as studied by Robinson[21] are well known—fibrillary contractions as seen in the ventricles, and small rapid contractions involving the major part of the auricular muscle, occurring at the rate of about 500

[21] Robinson: Journ. Exp. Med., 1913, xvii, 429.

per minute. In the present experiments this "*auricular tachycardia*" was also in evidence, but the extent of the accompanying *fibrillary contractions* was not determined by the method employed. The rapid contractions seemed coordinated, and of a constant rate, approximately 650 per minute. Their amplitude varied considerably, but in all cases was smaller than the amplitude of normal auricular systole.

The influence of these contractions is easily determined by stimu-

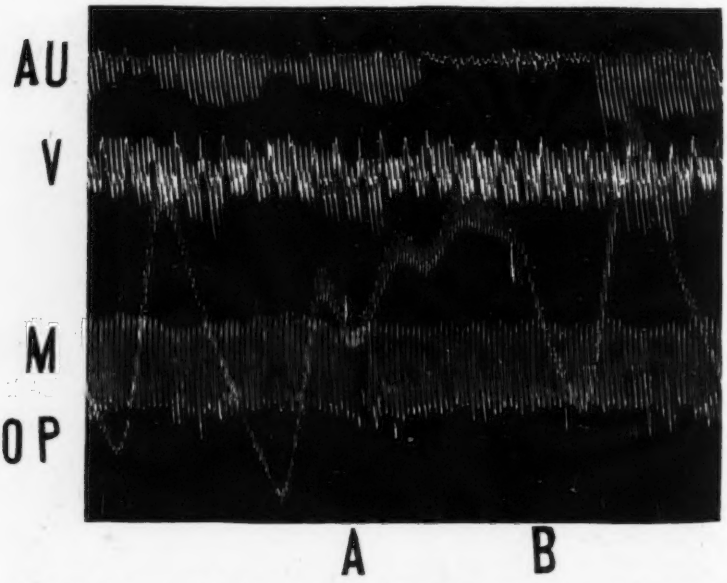

Fig. 17. A record showing very efficient fibrillary contractions of the auricles. Period of fibrillation *A-B*. *AU.*, Auricular contraction; *V.*, ventricular contraction; *M.*, myocardiograph; *O.P.*, volume output.

lating the auricules at the crest and at the trough of an interference wave see figure 15 and 16. In figure 15 the auricles are stimulated at the crest, when auricular systole is at its maximum efficiency. The output falls, but does not reach the minimum level maintained by venous pressure alone. In figure 16 the auricles are stimulated at the trough of the wave. In this case the output increases considerably over that maintained by venous pressure. In all cases these contractions had a very appreciable beneficial effect on ventricular efficiency,

and in some instances maintained an output very little below that maintained by combined venous pressure and auricular systole, properly placed. Such an instance is shown in figure 17. The period of rapid contraction is included between points (*A*) and (*B*). During this period there are small oscillations in the volume output tracing. These probably are due to rhythmical interference of the auricular and ventricular contractions.

The explanation of the effectiveness of these rapid contractions is suggested in figure 11. In this figure the lower is a ventricular myocardiograph tracing, the upper a left intraventricular tension tracing. (*A-B*) represents a period of rapid contractions (*B-C*) recovery to

Fig. 18. A record showing that the relative efficiency of fibrillary contractions of the auricles depend on the magnitude of normal auricular systole. *A-B*, Interference wave with normal auriuclar systole. *B-C*, Period of auricular fibrillation; *C-D*, Interference waves in which magnitude of auricular systole is increased. Upper tracing—Auricular contraction. Lower tracing—Volume output.

normal contraction. The upper tracing shows the development of intraventricular tension with each auricular contraction to be considerable. The effect of these contractions on ventricular volume appears in the myocardiograph tracing. Each steplike increase of volume-length in period (*D* to *E*) is the result of an individual auricular contraction. Compared with the period following (*E-F*) where auricular contractions are normal, the filling is quite as effective.

The relative effectiveness of the so-called fibrillary contractions as compared with the normal auricular contractions depends on two factors—the magnitude of the normal contraction and of the fibrillary

contractions, both of which can vary. Figure 18 illustrates this point. (*A* to *B*) represents the usual auriculo-ventricular interference wave, with the auricles contracting normally; (*B* to *C*) the auricles are fibrillating; at (*C*) the auricles recover and are contracting with increased intensity, the usual result of previous fibrillation. This record illustrates well the importance of magnitude of auricular systole as well as the importance of proper time relation.

EFFECT OF VAGUS STIMULATION ON VENTRICULAR EFFICIENCY

Stimulation of the vagus nerve with the heart in block effects the auricles primarily. By annullment of auricular systole in the course of an interference wave we might expect ventricular efficiency to fall approximately to that represented by the trough of the interference wave—the efficiency maintained by venous pressure alone. This obtains in figure 19. On stimulating the vagus at the crest of a wave,

Fig. 19. Effect of stimulation of the vagus nerve in the course of interference waves. *B.P.*, Arterial blood pressure recorded with the Hg. manometer; *AU.*, Auricular contraction; *T.*, Time in seconds.

the blood pressure falls rapidly to the level of the trough. But this sudden drop is followed by a small and gradual drop, the interpretation of which is more difficult. It may be explained by the observation of Erlanger[22] who finds that vagus stimulation with the heart in block has a small, but retarding effect upon ventricular rate. This same effect was noted in the present experiments and occurred apparently to the same degree whether the bundle was completely crushed or only temporarily pierced by the hook of the clamp. Erlanger noted further that the latent period of the retarding effect was longer in case of the ventricles than in case of the auricles. This could account for the delay of the second fall of pressure seen in the present experiments. Since ventricular rate was maintained constant in figure 19 by direct stimulation, the fall of pressure, if a direct vagus effect on the ventricles occurred, would be accounted for by effects other than chronotropic.

[22] Erlanger: Arch. f. d. ges. Physiol., 1909, cxxvii,

Other factors may likewise contribute to this fall of pressure—a more complete emptying of the ventricles progressively decreasing initial volume and thereby decreasing ventricular efficiency, or poorer ventricular nourishment resulting from the long maintained low pressure.

Figure 19 illustrates again the significance of magnitude of auricular systole. As auricular systole slowly recovers from vagus stimulation the magnitude of the blood pressure waves progressively increases.

SUMMARY

An attempt was made to determine the relation of ventricular efficiency to ventricular filling and to analyze and correlate the various effects of auricular contraction on cardiodynamics.

This was done with a modified heart-lung preparation of the dog, with the heart in block, by varying the magnitude of venous pressure and the effectiveness of auricular systole.

With the methods employed, rate of auricular and ventricular contraction, nature of auricular contraction, time relation of auricular to ventricular systole, venous pressure, and capillary resistance were under control.

By recording auricular and ventricular contractions, variation of length of ventricular fiber, which indirectly gives ventricular volume changes, right and left intraventricular tension, venous pulse, and left ventricular output, the effects of auricular systole were analyzed.

In connection with the methods used, a piston-myocardiograph, a differential volume recorder, a trocar-cannula and a pneumatic blood pump are described.

The importance of auricular systole was determined by the use of auriculo-ventricular interference waves.

Under the conditions of these experiments auricular systole increased ventricular output about fifty per cent over that maintained by venous pressure alone.

No definite statement can be made concerning the relative effect of auricular systole with different venous pressures. This depends largely upon duration of ventricular diastole, resistance of ventricular muscle to stretching, etc.

Reasons are given why the variations of ventricular efficiency in the course of an interference wave cannot be ascribed to disturbed valvular action.

Since even a moderately filled ventricle does not empty itself completely, ventricular volume per se cannot be the factor determining ventricular efficiency, but rather factors secondary to volume change.

Auricular systole means: (1) increased length of ventricular fiber, (2) increased initial intraventricular tension, and (3) altered surface-volume relation, all of which enhance ventricular efficiency.

1. Increase in length of fiber increases the strength of contraction by increasing the liberation of contractile energy. Duration as well as strength of contraction is markedly increased.

2 *a*. Increased initial tension increases the strength of contraction through the potential energy thereby stored in the ventricular walls. This potential energy is liberated as dynamic energy during systole and in turn helps to expel the blood.

b. This increased initial tension may also have a specific enhancing effect upon the processes of muscular contraction.

c. By minimizing initial waste contraction, and by tending to slow the early part of ventricular contraction, increased initial tension increases ventricular efficiency.

3. The changing surface-volume relation influences cardiodynamics in a number of ways:

a. It influences the effectiveness of a given muscle shortening by virtue of the fact that the volume of a sphere increases more rapidly than the surface; which means that the greater the ventricular volume the greater the output per unit shortening of muscle.

b. It increases the amount of liberated contractile energy by retarding muscle shortening during the early part of contraction, thereby, according to Hill, increasing the amount of liberated contractile energy.

c. It increases the efficiency of the available contractile energy in ventricular systole by virtue of the fact that the intraventricular surface over which the available energy is spread, decreases as systole progresses.

The manner in which the latter factor might play an important part in cardiodynamics is discussed.

Since the relative importance of the secondary factors vary in different proportions under varying conditions, since they affect each other in different ways, and are inseparably interrelated, it is impossible to allot to each its relative value.

The increase in ventricular volume noted on increasing cardiac demands is an adaptive reaction in which initial length of fiber, initial intraventricular tension, and surface-volume relation play a part.

The reaction suddenly breaks down when the counteracting factors become greater than the accompanying increasing strength of contraction.

Auricular fibrillation increases ventricular efficiency in a manner similar to auricular systole. Although the effects are not as marked, at times the so-called auricular fibrillary contractions are nearly as efficient as normal auricular contractions.

Stimulation of the vagus nerve, in the course of interference waves by annulling auricular systole results in ventricular efficiency approximately equal to that maintained by venous pressure alone.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXXIII. The Secretion of Gastric Juice in Cases of Gastric and Duodenal Ulcers

L. L. J. HARDT

From the Hull Physiological Laboratory of the University of Chicago

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No one has reported any direct and definitely controlled experiments with pure gastric juice either in clinical or in experimentally produced ulcers. It is commonly stated that in an ulcer of the stomach or duodenum the gastric glands undergo changes which result in any one of the following conditions: (1) Hypersecretion; (2) Hyperacidity; (3) Hyposecretion; (4) Hypoacidity.

Since this leaves the question of hyperacidity and hypersecretion in gastric ulcers unsettled, the present work was undertaken at the suggestion of Dr. Carlson, in the hope of securing data better controlled than is possible in man.

Pavlov (1) has reported one instance of spontaneous gastric ulcer in a dog with a Pavlov pouch. The ulcer lodged in the pouch, and he stated there resulted a hypersecretion but no hyperacidity. Stanley (2) concludes from eighteen cases of gastric ulcer that there is an actual increase in the acidity of the gastric contents but no hypersecretion. The maximum total acidity which he obtained was 0.40 per cent and the maximum free acidity was 0.35 per cent. The average total acidity was 0.31 per cent and the average free acidity was 0.32 per cent. Patterson (3) gives the following four analyses of gastric contents, which are typical of a number of cases: In duodenal ulcers distal to the pylorus, the total acidity is 0.32 per cent and the free is 0.02 per cent. In duodenal ulcers close to the pylorus, the total acidity is 0.3 per cent and the free 0.016 per cent. In gastric ulcers close to the pylorus the total acidity is 0.3 per cent, the free is 0.016 per cent and the protein HCl is 0.082 per cent. In gastric ulcers located in the middle of the stomach the total acidity was 0.321 per cent, the free is 0.01 per cent, and the protein HCl is 0.0824 per cent. He states, however,

that the analyses of the gastric contents alone, in the absence of the other symptoms of ulcer, has no diagnostic value. Rehfuss and Hawk (4) found certain deviations in the concentrations of the acidity and in the secretion curve in various pathological conditions. They make the statement that in most of the cases there is present a condition of hypersecretion but give few figures to substantiate such a conclusion. Christiansen (5), Michaelis and Davidson (6) determined the acidity of the gastric contents in cases of dyspepsia, gastric cancer, gastric ulcer and many other pathological cases. They found considerable variation in the acidity but in no instance was the acidity above 0.43 per cent. Neilson (7) makes the statement that hyperacidity is frequently encountered in cases of gall stones, floating kidney, hyperthyroidism, chronic appendicitis and ulcer of the stomach. Wolpe's (8) report is in direct contradiction to the statement of Neilson. He found achylia constant in all cases of pronounced types of exophthalmic goiter. Even when one of the classic triad of symptoms was lacking, the secretion of HCl did not seem to be modified.

The fact that clinicians are usually dealing with a mixture of juices (bile, pancreatic, salivary, and gastric) may account for the many contradictory results obtained in normal and pathological conditions. In no instance where hyperacidity is reported does the concentration of acid exceed 0.55 per cent. Boldyreff (9) in a recent review of the literature has shown that where almost pure normal human gastric juice was obtainable, the acidity expressed in per cent of HCl was from 0.35 per cent to 0.48 per cent. Carlson (10) and others have shown that normally in man the appetite juice has an average acidity of 0.45 per cent and may reach a total acidity of 0.55 per cent without showing any of the so-called symptoms of hyperacidity. Pavlov (11), Foster and Lambert (12), and many others have reported similar high concentrations in dogs.

The work done on the experimental production of gastric ulcers up to 1906 has been very thoroughly reviewed by Turck (13). Many workers have since been engaged in the production of gastric ulcers by other means. Friedmann and Hamburger (14, 15) have produced chronic and acute ulcers in dogs by tying a silk ligature loosely around the pylorus, thus producing partial pyloric stenosis, after which they injected 1 cc. of 5 per cent solution of silver nitrate directly into the mucosa. No cultures were made of the ulcers thus produced so that one is left in doubt as to whether the chronicity of such an ulcer is actually due to the impairment of motility, or to an infection of the area destroyed by silver nitrate. Before and after the production of the gastric

ulcer, test meals were given to the dogs, and after 50 minutes the stomach contents were removed and analyzed. In their tables they give figures showing a "hypersecretion" in some cases and a "hyperacidity" with a maximum total acidity of 0.3 per cent. They did not consider the factor of continuous secretion, so that what they called "hypersecretion," might be due to an excess of continuous secretion or a prolonged secretion as a result of stasis of food. Relatively few experiments were made on each dog and the data they publish might easily come within the normal variations in the volume and acidity in the gastric contents in any one dog. Their work meets with the same criticism as the work done on human beings, namely, in the analysis of the gastric contents, they do not deal with pure gastric juice.

Rosenow (16) produced chronic and acute gastric ulcers in dogs by intravenous injections of certain strains of streptococci. He showed that pure cultures of streptococci isolated from gastric ulcers in man, dog, cattle or sheep, when injected intravenously into dogs and rabbits, produced typical gastric ulcers in a large percentage of the experiments. Whether the chronicity of this type of ulcer is due entirely to the streptococcus infection or the combined action of the streptococci and the corrosive action of the gastric juice has not been proven experimentally. Sippy (17) has shown in his treatment of human gastric ulcer, that the gastric acidity appears to play an important rôle in establishing a chronic ulcer. In neutralizing the gastric juice by constant administration of alkalies he has effected a cure for a large number of chronic gastric and duodenal ulcers.

Steinharter (18) claims to have produced gastric ulcers quite uniformly in rabbits, by intravenous injections of 24-hour old broth cultures of B. coli agglutinated with 0.3 per cent hydrocloric acid for 24 hours. One cubic centimeter of the 0.3 hydrochloric acid is added to 2.5 cc. of the 24-hour old broth culture. The agglutinated B. Coli are washed with sterile normal salt solution and injected into the ear vein of the rabbit.

METHODS

1. Thyroid feeding

The conflicting reports of Wolpe, Nielsen and others suggested the possibility of producing hyperacidity and hypersecretion of gastric juice in Pavlov-pouch dogs by feeding thyroid. Although Carlson (19) has shown conclusively that it is impossible to produce all the typical symptoms of exophthalmic goiter in dogs by feeding desiccated thyroids,

the work was repeated with Pavlov-pouch dogs to determine the effects upon the gastric juice. Two dogs weighing 2.5 and 4 kilos, respectively, were fed 10 grams of Armour's desiccated thyroid daily. This thyroid feeding covered a period of two weeks. The collection of gastric juice was begun one hour before feeding and continued for one hour after feeding. The controls were made by feeding the same kind and quantity of meat in the absence of the thyroid. A series of controls were obtained before feeding the thyroid, another after the thyroid feeding was stopped.

2. Production of gastric and duodenal ulcers

a. Attempts with B. coli. In this series of experiments, the work of Steinharter was repeated on 18 rabbits and 3 dogs. The 24-hour old broth cultures of B. coli were agglutinated with 0.3 per cent HCl for 24 hours, using 1 cc. of the 0.3 per cent HCl to 2.5 cc. of the 24-hour old culture. The agglutinated bacteria were washed twice with sterile salt solution and injected intravenously into the animals. Five strains of B. coli were used.

Strain 1 was isolated from the normal faeces.

Strain 2 was isolated from a case of cholecystitis.

Strain 3 was grown on media containing normal salt.

Strain 4 was grown on media containing normal salt.

Strain 5 was a sub-culture of Strain 4.

Twelve rabbits were each given an injection of the growth from 5 cc. of a 24-hour old broth culture of B. coli agglutinated as above. Four of these rabbits were injected with Strain 4, four with Strain 3, and four with Strain 5.

Four rabbits were each given an injection of the growth from 10 cc. of a 24-hour old broth culture of B coli agglutinated as above. Two of these four rabbits were injected with Strain 1, and the other two with Strain 2.

Two rabbits were each given an injection of the growth from 10 cc. of a 24-hour old broth culture of B. coli which had not been agglutinated. One of these two rabbits was injected with Strain 1, and the other with Strain 2.

b. Ulcers produced by streptococcus. I followed Dr. Rosenow's technique in producing gastric and duodenal ulcers. My experiments, however, were chiefly on Pavlov-pouch dogs. In the first experiment the ulcer was produced by streptococci which Dr. Rosenow had isolated from a duodenal ulcer in a child. Later, at Dr. Rosenow's sug-

gestion and under his supervision, the streptococci were isolated from the gastric ulcers in sheep and cows. The best results were usually obtained by isolating streptococci from the muscular coats of an ulcer which had undergone very little healing. By this method the possibilities for contamination were minimized. The dogs were injected intravenously with the growth from 40 to 60 cc. of the ascites dextrose broth, the dose depending upon the size of the dog.

In three dogs an attempt was made to produce an ulcer in the pouch by injecting the streptococcus directly into and beneath the mucosa. This resulted in the immediate production of a pocket in the submucosa containing streptococci. To make sure of the presence of an ulcer as the result of the injection the following examination was made:

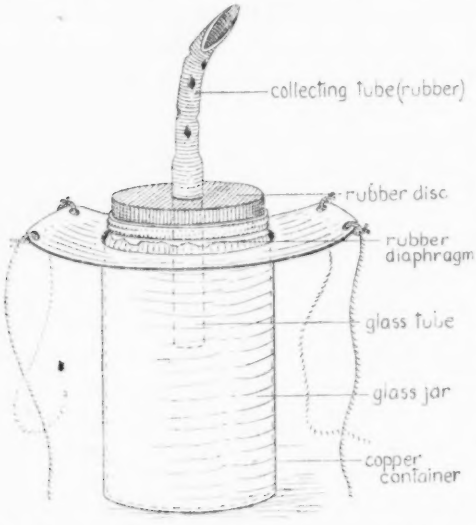

Fig. 1. Apparatus used in collecting gastric juice.

A small glass test tube with a movable mirror attachment at the bottom was introduced into the Pavlov-pouch of the dog. By throwing light into the tube sufficient illumination was obtained to enable one to examine the mucosa of the pouch. Three days after this injection there was no evidence of an ulcer by the examination described above.

c. Collection of gastric juice. The juice was collected in a small glass cup about 8 cm. high with a round base 5 cm. in diameter and a round top of 4 cm. in diameter. The cup was held in position by a slightly larger copper cup which fitted closely around the other cup. The copper cup had two small handles through which cords were passed and tied on the back of the dog. In this way the glass cup was held tightly in position against the abdomen of the dog. The glass cup had a tin screw top and lined with a thin rubber sheet which prevented any

leakage. A hole was bored in the center of the cover just large enough for the glass tube from the pouch to pass through it. By making the hole in the rubber sheet smaller than that in the tin cover the glass tube which drained the juice from the pouch was made to fit practically water tight. By means of such a mechanism (see fig. 1) the dog could walk about or lie down in his cage without spilling any of the juice. This method could not be employed if a dog persisted in lying on its back for in that case the gastric juice would leak around the tube and be absorbed by the bandage.

3. Manner of collection

The collection of gastric juice was made in the morning, care being taken to remove water from the cage at least one hour before beginning an experiment. In every instance but one (in that case the collection was begun one hour before feeding and continued one hour after feeding without removing the cups) the gastric juice was collected two hours before feeding and the volume, total acidity and free acidity was determined. The sample of juice collected before feeding will be called the "continuous secretion." The dogs were then fed a standard meal of 250 to 350 grams of ground, boiled beef moistened with water. The juice was collected at one hour intervals for two hours after feeding. The acidity was determined by titration with $\frac{N}{10}$ NaOH and using dimethyl amino azobenzene and phenolphthalein as indicators for the free and the total acidity respectively. The experiments were made on the dogs four to six times a week for at least two weeks and in some cases as long as four to eight weeks. After a sufficient number of control experiments were obtained they were given an injection of streptococci into the saphenous vein. Whenever a sufficient amount of streptococci were available, normal control dogs were injected with the same amount of streptococci that were given to pouch dogs. The control dogs were usually posted from 24 to 48 hours after an injection. If no ulcers were present in the normal dogs, the Pavlov-pouch dogs were later given a second injection. In one dog three injections of streptococci were made. The gastric juice was studied for varying lengths of time after the injection, that is from 2 to 8 weeks, in the same manner as it was studied before. The dogs were killed and then autopsied.

RESULTS

1. Thyroid feeding

The thyroid feeding experiments were discontinued at the end of the second week because there was no indication of either hyperacidity or hypersecretion. In both dogs on the contrary, there was a tendency toward depression of the acidity and the rate of secretion as is shown in Table 1. The dogs were in perfect health throughout the experiment, and from all appearances the thyroid had no toxic effects; they ate readily and so far as could be judged there were no gastro-intestinal disturbances. The acidity and volume of the gastric juice returned to normal a few days after the thyroid feeding was discontinued.

2. B. Coli

Of the eighteen rabbits injected with B. coli, negative results were obtained in all but four rabbits. In the rabbits where lesions were produced, all were injected with Strain 4. The results of the injection with Strain 4 are as follows:

Rabbit I died in less than four hours after the injection. Autopsy performed immediately after death. The stomach was perforated at the fundus. There were ulcers and hemorrhages near the pyloric end. The intestines were quite normal. The ulcers were not the typical round ulcers. The mucosa appeared to be sloughed off in places. The muscular coats were normal so that it was deemed useless to attempt to make a pure deep culture of the ulcerated area.

Rabbit II died in less than 24 hours after the injection. Autopsy: There was an ulcerated area near the fundus; intestines showed signs of hemorrhage.

Rabbits III and IV had hemorrhages in the stomach and slightly hemorrhagic areas in the duodenum. Both were killed 48 hours after the injection. No typical round ulcers were found.

In all three dogs the strain (1, 2, 3) of B. coli proved highly toxic; the dogs died in less than 24 hours and no specific lesions were found. In one of the dogs there was considerable hemorrhage in the duodenum and stomach.

3. Production of gastric ulcer by injection of streptococci

Ulcers were produced by injections of streptococci isolated from ulcers in man, sheep, cattle and dogs. The ulcers produced may be divided into the "acute" and "chronic" type of ulcer. The term

TABLE I

The influence on the secretion of gastric juice, of excessive thyroid feeding experimental "Hyperthyroidism."

CONDITION OF DOG	NUMBER OF EXPERIMENT	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dog I.										
One hour before and one hour after feeding 250 grams meat	20	18	8	13.15	0.4922	0.3737	0.4183	0.4649	0.3281	0.3644
Feeding 250 grams meat and 10 grams of thyroid. One hour after and one hour before feeding	10	16	3.3	8.2	0.3463	0.1915	0.3068	0.3646	0.1459	0.2674
Stopped feeding thyroid, one hour before and one hour after feeding	12	14	8	10.8	0.4922	0.0820	.05481	0.4102	0.0450	0.3126
Dog II.										
One hour before and one hour after feeding 300 grams meat	10	13	7	9.09	0.4010	0.1820	0.3047	0.3646	0.1361	0.2532
Feeding 300 grams meat a and 10 grams of thyroid. One hour before and one hour after feeding	12	13	2	6.5	0.3646	0.0000	0.1139	0.3281	0.0000	0.0625
Thyroid feeding stopped. One hour before and one hour after feeding	8	16	5	10.6	0.4193	0.1704	0.3281	0.3593	0.0656	0.2372

acute as applied here is an ulcer which is present in an active progressive state ten days to three weeks after an injection. The chronic ulcer is one which is active five to eight weeks after an injection. With one exception the ulcers resulting from the streptococcus injection lodged in the Pavlov-pouch. In Dog 7 a chronic ulcer was formed in the duodenum, about 8 cm. from the pylorus. Twelve normal Pavlov pouch dogs were examined for gastric ulcers but no ulcers were present. Spontaneous gastric ulcers were found in two Pavlov-pouch dogs not injected with bacteria, and in one Pavlov-pouch dog with complete pancreatectomy. At atopsy cultures were made from most of the ulcers and pure cultures of streptococci were isolated. The virulence of the isolated streptococci was demonstrated in two cases by injections into two dogs. Gastric ulcers with hemorrhages of the stomach followed the injections, and both dogs died in less than 24 hours. Gross and microscopic examinations were made in every case and active progressive ulcers were demonstrated.

Ulcers were produced in eight cases out of the six normal dogs and eight Pavlov-pouch dogs. Positive results were obtained therefore in 57 per cent of the experiments, seven of those successful attempts were in Pavlov-pouches.

DISCUSSION

The thyroid feeding experiments are not sufficient in number to warrant a conclusion as to the cause of the depression in volume and acidity of gastric juice during the first hour after eating. Further work should be done to determine the cause of the depression and whether or not the depression involves only the secretion of the appetite gastric juice or the entire secretion curve. To determine this it would be necessary to follow out the secretion during the entire course of digestion. The depression is not permanent as is shown by the rapid return to normal acidity and secretion rate when the thyroid feeding was stopped.

The experiments with B. coli do not confirm the work of Steinharter. In only one series of experiments was there any indication that intravenous injections of B. coli in rabbits (by Steinharter's method) would produce gastric ulcer. Five different strains of B. coli were used, but only one strain appeared to be toxic to the rabbits' stomach and gastric *lesions* were produced in every one of the four rabbits injected with that strain (Strain 4). There is in all probability a difference in the virulence and specificity of the toxins produced by B. coli. But the

gastric lesions resulting from intravenous injections of B. coli are not the typical ulcers which can be produced by streptococci.

The productions of gastric ulcers in dogs by intravenous injections of streptococci which were isolated from gastric ulcers in man, dogs, cattle and sheep, is a confirmation of Rosenow's work. The fact that the ulcers lodged (in the majority of cases) in the Pavlov-pouch of the dogs, demonstrate many points of interest which undoubtedly are of some clinical significance. The intensity of the movements and contractions of the Pavlov-pouch is about as vigorous as in the fundus of the main stomach. Since there was no obstruction in the pouch to alter the motor mechanism, the chronicity of the ulcers must be explained on some other basis. The acidity of the gastric juice was not materially increased in any of the dogs and only two of the dogs with ulcers showed a hypersecretion.

One is inclined to lay less stress upon the factors of acidity, secretion and mechanical obstruction when we see active chronic ulcers produced in the Pavlov-pouch. In the pouch the motility is less than in the pylorus, there is practically no mechanical irritation, and the gastric juice is constantly being drained from the pouch. Since the mechanical factors and the corrosive action of the gastric juice is practically eliminated, the chronicity of the ulcer must depend primarily upon the virulence of the organism producing the ulcer. This is further demonstrated in the Pavlov-pouch operation. There is considerable destruction of the mucosa in a Pavlov operation; but since the necrosed mucosa is not infected, healing takes place in a few days in spite of the "corrosive action" of the gastric juice. The production of the gastric ulcers and the chronicity of the ulcer is dependent primarily upon the virulence of the streptococci infection. The haematogoneous origin of the infection is further demonstrated in the unsuccessful attempt to produce chronic ulcers by local injections of streptococci into the submucosa of the Pavlov-pouch.

The striking variations in the secretion rate and acidity which each dog showed after a test meal will probably explain some of the conflicting clinical data. The results were obtained from seven dogs with gastric and one with a duodenal ulcer (figs. 2 and 3). There was no change in the concentration of the acidity which indicated the presence of an ulcer in any one of the dogs. The acidity ranged from 0.0000 per cent to 0.55 per cent both before and after the production of the ulcer. There was a depression in the acidity of the gastric juice [illegible] Dog III. In two of the dogs (Dogs IV and II, Table II) there was a tem-

porary continuous hypersecretion and a hypersecretion after eating but the acidity was about normal.

In Dog II there was a hypersecretion in spite of the fact that she had developed distemper a few days after the injection. Distemper in a majority of cases depresses the acidity and secretion. Why only two of the eight dogs should develop a hypersecretion we are not at present able to satisfactorily explain.

This hypersecretion may have been the result of an increased sensibility of the gastric mucosa either produced locally or as a result of the absorption of toxins from the ulcer and a stimulation of the vagus fibers. In gastric ulcers involving extensive areas of mucosa it is quite conceivable that a hypersecretion might result from the formation and absorption of gastrin. To determine whether the hypersecretion is due to a local stimulation, or reflex, it would be necessary to perform a series of similar experiments on dogs with Heidenham pouches in which the vagus fibers going to the pouch have been severed.

CONCLUSIONS

1. Feeding excessive amounts of desiccated thyroid depresses the rate of secretion and concentration of acidity in the gastric juice during the first hour after feeding.

2. The results of Steinharter ("The production of acute ulcers in rabbits by intravenous injections of B. coli") have not been confirmed.

3. Gastric and duodenal ulcers can be produced in dogs by intravenous injections of streptococci isolated from gastric ulcers in man, dog, sheep and cattle. This is a confirmation of the work of Rosenow.

4. There is no "hyperacidity" in the gastric juice following the experimental production of gastric ulcers.

5. Gastric and duodenal ulcers may or may not result in a continuous hypersecretion together with a hypersecretion after eating.

I wish to thank Dr. Rosenow under whose supervision the bacteriological work was carried out; and Dr. Carlson for his suggestions.

PROTOCOLS

Dog I.

Experiments were begun June 8 and continued until August 19, 1915.

Two injections of streptococci, which I isolated from ulcers in sheep were made directly into the mucosa and submucosa of the Pavlov pouch. No ulcers resulted from the injection.

July 19. Injected the growth from 40 cc. of ascites-dextrose broth of streptococci isolated from an ulcer of a cow.

Injected a similar dose into a normal dog of about the same weight. Following the injection of streptococci, both in the normal and in the Pavlov dog; the dogs vomited a bile colored fluid. Depression followed the vomiting and lasted for 5 to 6 hours. The day after the injection the dogs were quite active.

July 21. Posted the normal control dog and found an ulcer in the pyloric end of the stomach. The ulcer was about 5 mm. in diameter.

The Pavlov dog was in perfect condition during the entire experiment.

August 20. She was chloroformed; autopsy showed her to be quite normal except for a single elongated ulcer in the small pouch. The ulcer was 2 cm. long and 4 mm. in the widest portion. The edges of the ulcer were rounded and slightly undermined. The base was smooth and fibrous. The extremities of the ulcer were actively progressing. The edges of the extremities were elevated and hyperaemic.

Dog II.

Experiments covered a period of 44 days, beginning April 14 and ending May 27. Two weeks were allowed for the dog to recover from the effects of the Pavlov operation before beginning the experiments.

May 7. Injected streptococci, isolated by Dr. Rosenow from a gastric ulcer in a hog, directly into the submucosa of the small pouch.

May 9. No ulcer was found at the site of injection. Examination was made by introducing a small test tube with a mirror inside, into the pouch. By this method one could carefully study the mucosa of the pouch.

May 18. I injected intravenously with the growth of 40 cc. of ascites-dextrose broth of a strain of streptococci which I isolated from a gastric ulcer in a sheep.

May 20. She ate very heartily but secreted slightly more juice than her maximum secretion up to this time. The juice was blood tinged.

May 21. The dog does not eat very much and secreted a few cubic centimeters of blood tinged juice.

May 24. Dog shows beginning distemper. She refused food and water. In the absence of food or water there was a continuous hypersecretion with an acidity of approximately 0.3 per cent. The stomach was aspirated but no food was presented.

May 25. Condition about the same as May 24.

May 26. Same as May 25 but more mucous was secreted.

May 27. Only mucous was secreted.

Killed her. Autopsy: Slight lesion in the heart, all the other organs are normal. A large irregular ulcer, 1.5 by 2 cm. was found a little to one side of the suture line in the small pouch. The mucosa was completely necrosed but had not sloughed off as yet.

Culture showed mainly streptococci with a few B. coli. Microscopic examination showed active progressive ulcer which had extended into the muscular coats. There was a slight scar tissue formation. Leucocytic infiltration had extended into the muscular coats.

Dog III

The experiments on this dog covered the period from April 9 to August 26.

May 6. Injected streptococci, isloated from gastric ulcer in a sheep, directly into the submucosa. No ulcer was produced.

May 19. Injected the growth from 10 cc. of ascites-dextrose broth of streptococci, isolated from a gastric ulcer in a sheep, directly into the submucosa. No ulcer was produced.

May 23. Injected the growth from 60 cc. of ascites-dextrose broth of a 24-hour culture of streptococci isolated from an ulcer in a sheep. The same dose was injected into another Pavlov pouch dog and killed a week later. No ulcer was found.

One normal dog and a normal rabbit were injected with the same dose and 58 hours later were killed. The dog had a single small ulcer in the pyloric end of the stomach. The rabbit had a small ulcer near the fundus of the stomach.

Dog VII, immediately after the injection, had violent vomiting movements which were followed by marked weakness. The dog recovered completely by the following day.

The dog appeared in perfect health with the test meals and laboratory treatment up to the time she was killed (August 27).

Autopsy: All the organs were normal; the stomach, including the Pavlov pouch, was quite normal.

There was a single round ulcer about 1.5 cm. in diameter located 6 cm. from the pylorus in the duodenum. The margins of the ulcer were elevated and undermined. The base of the ulcer was hard and smooth.

Dog IV.

Operated for Pavlov pouch about six months previous to injection. She was injected February 16, with the growth of from 25 cc. of ascites-dextrose broth of a strain of streptococcus isolated by Dr. Rosenow from a duodenal ulcer of a 12 year old child. There was vomiting and great weakness following the injection. This condition lasted for about 2 hours.

February 17. The dog seems fairly well, eats heartily but secretes practically no juice during the first 2 hours after feeding. This condition continued for one week after the injection. During that time the juice (which was collected for one hour before and two after feeding) consisted of a thick bloody mucous practically free from acid.

February 25. A clear juice was secreted; both before and after feeding. Following the sudden change from the bloody mucous secretion to the clear juice that resulted in a slight increase in the average volume of gastric juice amounting to 3.1 cc.

There was a continuous hypersecretion which lasted for six days and then returned to normal. The dog continued in an apparently normal condition until March 1, when she began to show muscular tremors, slight stiffness of the joints and great weakness.

March 1. The dog died and was autopsied immediately.

Autopsy: There was hemorrhage in the small intestine, and a coffee ground colored fluid in the stomach. The mucosa of the stomach was normal except

for a round pouched out ulcer in the pouch, 2 cm. from the suture line; the ulcer was 12mm. in diameter. The margins of the ulcer was thickened and hyperemic. The center of the base of the ulcer showed scar tissue. Microscopic examination —There was marked leucocytic infiltration which extended into the submucosa and muscular coats. Culture made by Dr. Rosenow showed a great many streptococci and a few B. coli.

The streptococci was injected into two other dogs and produced ulcers in both dogs. The dogs died in less than 24 hours of hemorrhage of the intestine and stomach.

Dog V

Pavlov pouch operation was performed 2 weeks before beginning the experiments. The experiments covered a period of three months, beginning November 24 and ending February 24.

January 6. The dog received an injection of streptococci, isolated from an ulcer by Dr. Rosenow. Control experiments showed the strain to be of a low grade of virulence.

January 28. Streptococci, which were isolated from a gastric ulcer, were injected intravenously. Following the injection the dog vomited and appeared very weak for several hours.

January 30. The dog is lame—the hind legs are somewhat stiffened.

February 22. Noted that food passed through the main stomach into the pouch.

Febuary 24. The dog was killed and an ulcer was found at cap of the small pouch. The ulcer had perforated so that there was a direct communication between the large and small pouch.

Cultures showed many streptococci, a few B. coli. Microscopic and gross examination showed an active progressive ulcer. The ulcer was about 6 mm. in diameter. The margins were elevated and slightly undermined.

Dog VI

Experiments covered a period of 2 weeks, beginning March 31 and ending April 15. The data is given in the table. No injection of streptococci was made in the dog.

April 10. The dog began to show signs of distemper.

The main stomach was markedly hemorrhagic.

The pouch appeared quite normal except for a small round, deep ulcer situated in the middle of the pouch and some distance from the suture line. The base of the ulcer showed some fibrous tissue. The edge was slightly undermined showing the ulcer to be quite active and progressing. This was a spontaneous ulcer evidently of streptococci origin as culture showed practically all streptococci.

In the dog the ulcer was evidently present from the time the experiments were begun. There was no evidence of a continuous hypersecretion or hyperacidity. The case is similar to that of Dog III. Both dogs developed sniffles and both dogs had ulcer in the pouch—in the one case there was no evidence of continued hypersecretion (Dog IV) while in the other there was a temporary continuous hypersecretion (Dog III).

Dog VII

Operated July 9, 1915; completely healed by July 13. Experiments were begun on her July 13 and continued until August 9, when she was killed. She appeared perfectly normal and ate readily until July 23. She refused food for 2 days but by July 29 she had recovered and was again quite normal.

August 4. She began to show symptoms of distemper.

August 5–8. The symptoms of distemper are more prominent. Tried to "force feed" her but she could retain nothing on her stomach.

August 9. Killed. Autopsy: Heart, lungs, kidney and spleen normal. Found partly healed round ulcer (about 6 mm. in diameter) in the small pouch. The main stomach had one small ulcer which was almost healed. There was an acute gastritis of the main pouch; the small pouch was quite normal except for the ulcer. The ulcer in the small pouch was active and progressive. The margins were more or less slightly undermined and infiltrated.

RESULTS

The acidity and rate of secretion of juice with gastric ulcer

In Table II is given the data on the dogs in which gastric ulcers were produced, either experimentally or spontaneously, together with the data which is typical of a number of the dogs in which no ulcers were produced and which are designated normal dogs. One can readily see from an examination of the data for "normal dog" that there is considerable variation in the acidity and rate of secretion of individual dogs and in the same dog even when a standard meal is given. In the table including the work done on the dogs with ulcers, the same wide variations can be seen both before and after the production of the ulcers. There were only two dogs in the series in which there was any indication of a change in the nature of the juice. Dogs IV and II both showed a tendency toward continued hypersecretion which lasted for a few days and then returned to normal. In *no* case was there any change in the acidity of the gastric juice which could be considered a "hyperacidity." In Dog III there was a slight depression in the acid concentration. There were variations and fluctuations in the rate and acidity of the gastric juice during the course of the experiments which one is bound to consider as normal variations.

TABLE II

The gastric juice in experimentally produced gastric and duodenal ulcers

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dog. I, Normal										
Two hours continuous secretion	15	4.5	1 drop	2.5	0.0450	0.0000	0.00478	0.0364	0.0000	0.0024
First hour after feeding	15	5	0.5	2.84	0.2462	0.0458	0.1415	0.2280	0.0000	0.1080
Second hour after feeding	10	7	1.5	4.5	0.4375	0.1368	0.2907	0.4010	0.0547	0.2409
Dog I, Gastric Ulcer										
Two hours continuous secretion	15	5	0	2	0.0912	0.0000	0.0072	0.0364	0.0000	0.0024
First hour after feeding	14	3.5	1	2	0.2188	0.0000	0.1324	0.1915	0.0000	0.0863
Second hour after feeding	14	6.5	1	3.2	0.4375	0.820	0.3034	0.4010	0.0364	0.2679
Dog II, Normal										
Two hours continuous secretion	16	4	1	2.28	0.2462	0.0000	0.0176	0.2006	0.0000	0.0143
First hour after feeding	15	7	2	3.45	0.3737	0.0000	0.1778	0.3372	0.0000	0.1432
Second hour after feeding	11	7	1	4	0.4010	0.1641	0.3320	0.3554	0.1276	0.2942
Dog II, Gastric Ulcers										
Two hours continuous secretion	8	10	2.5	6	0.3646	0.0000	0.2301	0.3190	0.0000	0.1892
First hour after feeding	5	9	2	4	0.3281	0.2097	0.2712	0.2736	0.1550	0.2077
Second hour after feeding	4	13.2	2	7	0.4284	0.2097	0.3524	0.4010	0.1550	0.3129
Dog III, Normal										
Two hours continuous secretion	25	11	0.5	2.03	0.4193	0.0000	0.0550	0.3919	0.0000	0.0460
First hour after feeding	14	10	2.25	5.7	0.4375	0.2462	0.3365	0.4102	0.2006	0.3043
Second hour after feeding	10	8	1.5	5.1	0.5287	0.3919	0.4474	0.5014	0.3646	0.4206
Dog III, Duodenal Ulcer										
Two hours continuous section	26	4.5	0.5	2.4	0.2644	0.0000	0.0155	0.2371	0.0000	0.0102
First hour after feeding	35	6.5	1.5	3.2	0.3463	0.0364	0.1704	0.3180	0.0000	0.1375
Second hour after feeding	24	7.75	1	3.8	0.4193	0.1459	0.3176	0.3828	0.0547	0.2742

TABLE II—Continued

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dog IV, Normal										
Two hours continuous secretion.....	14	11	2	3	0.4193	0.0000	0.0656	0.3737	0.0000	0.0110
One hour continuous secretion combined with one hour after feeding.....	20	13	7	9.09	0.4010	0.1820	0.3047	0.3646	0.1361	0.2532
Two hours continuous secretion combined with two hours after feeding.	15	25	5	16.7	0.4570	0.2372	0.3593	0.4272	0.1640	0.3126
Dog IV, Gastric Ulcer First Week										
Two hours before and two hours after feeding.....	7	6	1	3.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Second Week										
Two hours before and two hours after feeding.....	6	32	11.5	19.6	0.3737	0.2553	0.2904	0.3463	0.1368	0.2468
Two hours continuous secretion.....	6	11.5	4.5	8.2	0.3919	0.1915	0.2862	0.3463	0.1368	0.2506
Dog V										
One hour before and one hour after feeding.....	20	18	8	13.15	0.4922	0.3737	0.4183	0.4649	0.3281	0.3684
One hour before and one hour after feeding.....	12	14	8	10.8	0.4922	0.0820	0.3481	0.4102	0.0450	0.2893
Dog VI, Spontaneous Ulcer										
Two hours continuous secretion.....	8	4.5	0.5	2.1	0.1294	0.0000	0.0258	0.0642	0.0000	0.0128
One hour after feeding.....	8	7	2.5	4.1	0.3007	0.1550	0.2081	0.2644	0.1276	0.1778
Second hour after feeding.....	6	7	0.5	3.4.	0.4193	0.1641	0.2780	0.4041	0.1185	0.2435
Dog VII, Spontaneous Ulcer										
Two hours continuous secretion.....	10	6	0	2.1	0.1820	0.0273	0.1194	0.1276	0.0000	0.290
First hour after feeding.....	10	10.5	1	4.9	0.4102	0.2553	0.3208	0.3463	0.1820	0.2661
Second hour after feeding.....	10	12.5	1.5	6.2	0.4740	0.3281	0.4128	0.4465	0.2462	0.3709

TABLE II—Continued

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Normal Dog I										
Two hours continuous secretion	14	6	1	2.3	0.2006	0.0000	0.0196	0.1276	0.0000	0.0175
One hour after feeding	14	9	2	5.3	0.4010	0.0734	0.2636	0.3646	0.0547	0.2195
Two hours after feeding	14	12	2.5	6.1	0.4508	0.3281	0.4001	0.4284	0.3007	0.3685
Normal Dog II										
Two hours continuous secretion	5	6	4	4.04	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
One hour after feeding	5	7	4	5.4	0.2736	0.1276	0.1987	0.2553	0.0912	0.1641
Second hour after feeding	5	5	3.5	4	0.3007	0.2097	0.2507	0.2736	0.1820	0.2256
Normal Dog II										
One hour before and one hour after feeding	10	14	4	7.6	0.3737	0.1276	0.2673	0.3281	0.0364	0.2066

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BILE PIGMENT METABOLISM

I. Bile Pigment Output and Diet Studies

C. W. HOOPER, and G. H. WHIPPLE

From The George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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In the following communications we propose to show that the bile pigment secretion can be influenced at will by modification in the diet. This means probably that the liver has a *constructive* function in forming bile pigments as well as the accepted eliminative function which depends on the destruction of red cells containing hemoglobin. The statement that bile pigment elimination may be influenced by dietary conditions is proved conclusively for dogs, and there is every reason to suppose that it is true for other animals.

We realize that the above statements are contrary to the accepted views of physiologists, and will require very convincing proof which we submit in detail below. The generally accepted theory covering the life history of the bile pigments may be sketched somewhat as follows. Degeneration of red cells frees hemoglobin which is brought to the liver and there changed to bile pigments which are excreted as waste products into the intestine. Here the bile pigments are reduced to urobilin or stercobilin some of which may be absorbed and returned to the liver and again thrown out in the bile or destroyed. Some of this urobilin may escape the liver and appear in the urine, especially when the liver is not functioning normally.

We hope to add several factors to this relatively simple equation which may throw more light on the functional capacity of the liver as well as the cycle of pigment metabolism in the body. We have established (12) the fact that hemoglobin can be rapidly changed to bile pigment in the body circulation outside of the liver. McNee (5) has recently confirmed this observation. We (4) have also shown that the pleural and peritoneal cavities can rapidly transform hemoglobin into bile pigment. We believe that this extrahepatic transformation of hemoglobin into bile pigment may be more important than is generally

supposed, particularly in diseased conditions associated with icterus or hemoglobinemia.

We (11) have recorded the observation that an Eck fistula dog with obstructed common bile duct will develop icterus to a much less degree than a normal dog with obstructed duct. It has been proved that an Eck fistula liver is smaller than a normal liver and has less functional capacity (13 and 14). The suggestion is obvious that the production of bile pigments is in part due to the functional activity of the liver and not solely to the hemoglobin destruction. In this manner were we lead into a study of bile secretion and its many difficult problems.

An immense amount of work has been done upon the secretion of bile, and it is fair to say that most of it was not done in a critical spirit but apparently with the idea of proving that some one or more substances were cholagogues. Stadelmann (7 and 8) and his co-workers are among the few who emphasize the normal variations in bile flow in dogs and the extreme care necessary in drawing any conclusions from small fluctuations in bile excretion. Their experimental observations are carefully made, suitable controls are furnished, and great care is used in the analysis of figures. Their work is to be recommended as an example for all workers in this difficult field.

"Bile circulation" has been definitely established—Stadelmann (9). This means that the *bile salts* are poured into the intestine, partially absorbed from it and again excreted in the bile. This fact comes in with the observation that bile or bile salts are active cholagogues, in fact the only substances upon which there is agreement among experimental workers. Among the dozens of other drugs used as cholagogues one can pick out any single drug and find in the literature several workers who claim to show that it is a cholagogue and again the same number who claim as proven from their work that it has no cholagogue action. Almost all of the conservative workers agree that whole bile or bile salts alone give definite acceleration to the flow of bile.

"Bile circulation" for the *bile pigments* has been claimed but never demonstrated. Stadelmann (9) says that a very small amount of bile pigment *may* be absorbed from the intestine but there is no definite proof for this, and he leaves the question open. There is no question that the liver can pick bile out of the circulating blood and rapidly excrete it through the gall ducts. This applies to foreign bile as Wertheimer (10) showed by means of sheep's bile injected into dogs. This, however, does not prove that bile pigments can be absorbed from the *intestine* and excreted again in the bile as is assumed by some writers.

Hemoglobin injected into the blood stream, peritoneum or subcutaneous tissues will cause a rise in bile pigment output from the liver. Stadelmann and his pupils submit the best experiments on this point, but they do not claim that it is quantitatively eliminated as do Brugsch and Yoshimoto. The published data of Brugsch and Yoshimoto (2), Brugsch and Kawashima (3) do not establish their claims that hematin is quantitatively eliminated as bile pigment. But grant for the sake of argument that hematin *is* quantitatively eliminated as bile pigment, one is surprised at their argument therefrom that hemoglobin is the source of the bile pigment, and from the bile pigment one can compute the life cycle of the red cells! There may be a dozen substances which may be quantitatively or partially changed into bile pigments or the liver cells may be capable of building up bile pigments from various "building stones."

Some work has been done upon the bile pigment excretion as influenced by the injection of hemoglobin, bile and bile pigments, also various poisons known to injure the liver or destroy red blood cells (Stadelmann, Brugsch, Wertheimer, etc.). So far as we know no worker has followed the curve of bile pigment excretion with suitable control of general condition and weight of dog, hemoglobin estimation and red cell counts, the presence or absence of pigments in the urine and above all, the diet. For the present we shall confine our attention to the bile pigment output with occasional notes concerning the volume of bile flow.

At the beginning of this work about three years ago it seemed quite necessary that the bile fistula dogs should be maintained as near to a normal healthy condition as possible. Considerable time was spent upon this point, and every sort of ration was tried out, mixtures given with fresh bile and dried bile, raw liver and cooked liver, raw and cooked meat, milk, raw eggs, butter, fats, etc. We do not wish to dwell upon negative results, but will give only a review of our positive results.

We are convinced from our work that bile is a necessary life factor for a dog fed upon any common mixed diet. There are statements in the literature Albu (1), Ransom (6) that bile secretion is not essential to health in man, but we are sceptical of such reports as careful autopsy records are not submitted. We are convinced from our series of over twenty animals that it must be very unusual for a dog to be able to survive on any ordinary diet if the bile is *completely* excluded from the intestine. In our experiments, the bile which the animal may lick from its fistula during the night is not able to maintain normal equilibrium or anything approximating it.

We wish to point out again that the common bile duct can reestablish its lumen after double ligation with silk and resection of about 1 cm. of the duct. We have reported such cases in another paper (11), and note also that this can happen in a dog with a bile fistula. A tiny fistulous tract may establish itself between the cut ends of the common duct and allow the escape under pressure of a small amount of bile into the duodenum. One such dog is included in our series of simple bile fistulas. This point must be kept in mind and the stools watched very carefully for stercobilin and at autopsy a very careful search made at the site of the section of the common bile duct. When a dog with a permanent bile fistula on an ordinary diet holds his normal weight and condition, we believe that this possibility should be considered and excluded or not by examination of the feces. Only a very small amount of bile seeping into the duodenum is required to completely change the clinical picture of wasting and general malaise to one of health and activity. Study of these repaired common ducts at autopsy shows how small an amount of bile introduced at this point is necessary for health. Introduction of bile by stomach tube, however, has no such favorable effect.

Fresh bile (pig) was tried in two bile fistula dogs with poor success. It was given once or twice daily by stomach tube. This did not prevent the usual loss of weight on a mixed diet. Recently we have had better results using fresh dog's bile mixed with the food, but some animals will not eat this mixture, and it does not have permanent effects when given once or twice daily in 25 to 50 cc. amounts by stomach tube.

Dried ox bile (two grams per day) was given in capsules to many of the dogs in our earlier experiments. This dried bile surely helps maintain a normal condition, but as a rule is not sufficient with a simple mixed diet, and most of these bile fistula dogs died after several weeks with the familiar picture of emaciation, intestinal disturbances, including much loss of blood, and stupor. Dried ox bile cannot be counted upon to replace in any satisfactory way the normal flow of bile, but it does some good in some instances.

Fresh pig's liver was tried as a diet following quite a series of unsuccessful bile fistulas which were fed bile in various ways. Marked improvement was noted with fresh liver forming a part of the diet, but the dogs soon refused to eat the raw liver. Cooked liver was then tried with the same success, and we feel very certain that bile fistula dogs can be kept in practically normal condition and weight equilibrium

on a diet of cooked liver (pig or sheep). This statement must be slightly qualified as some dogs do not react as well as the majority: again the dogs' condition may remain perfect for months with a terminal loss of ground and intoxication. The diet used in the majority of experiments was the usual mixed diet of cooked meat, bones, and bread plus 100 to 200 grams of cooked liver as indicated in the charts.

That liver feeding in dogs with complete bile fistulae is of peculiar benefit, and may maintain them in a normal condition for weeks is pretty definitely established. This liver feeding is more efficacious in most cases than feeding fresh or dried bile, and is to be considered in the treatment of certain clinical cases. What particular chemical substance in the liver is responsible for this influence on the abnormal metabolism of bile fistula dogs? We hope to give an answer to this question in the near future.

METHODS

1. Operation and post-operative care

The operative procedure and care of the animals are very important factors in these experiments, and have not been sufficiently emphasized by many workers. The object should be to maintain the dog in as near to perfect condition as possible, and this is not easy.

All operations are done under ether-morphia anaesthesia, and strong, active, short-haired dogs of about thirty pounds weight are most suitable. An incision is made in the mid line, and the gall bladder dissected free from the liver. The common duct is freed, doubly ligated, and about 1 cm. between the ligatures is resected. The gall bladder is then pulled through a small stab wound in the right rectus close to the costal morgin, and fixed by silk sutures to the sheath of the rectus. The stab wound should be rather small, as a small fistula is desired. The gall bladder is then opened, and a small piece of rubber tubing about 1 cm. in diameter is pushed down into its lumen, and fixed here by two stay sutures. The median incision is closed as usual. No dressings are applied, as they only serve to irritate the skin, and the wounds heal very promptly. The tube in the gall bladder should be removed on the sixth or eighth day, and care should be taken that the tube drains freely, else a distinct icterus may result, and prolong convalescence or actually render the animal useless for further work. If there are no complications, the dog should be in good condition by the third week, and ready for bile collections by the method described below.

A regular routine is very important in the experiments, and is a part of the care so necessary to keep the dogs in good condition. The dogs are allowed to exercise in a yard for about one-half hour in the morning. They are brought in for collection of bile about 10 a.m., and put up in the harness for a period of six or eight hours, during which time specimens are removed every two hours. The dogs are fed two hours after the start of the experiment each day. At the end of the observation they are turned into the yard to exercise about one hour, and then given a heavy feed and locked in their cages. Dogs and cages are kept very clean, and dogs are washed once or twice a week. Collections are made *every* week day, and this is important, because otherwise the fistula will narrow and obstruct the outflow, jaundice will supervene, and the general condition of the dog will suffer. Great variations in the bile pigment output will then occur. It is best to dilate the bile fistula occasionally but very gently. On Sundays the fistulas are drained by a catheter, but no collections are made. Under this régime, the bile collected will be perfectly clear, except for an occasional small shread of mucus, and at autopsy the larger bile passages will be smooth, pale and normal throughout except for slight dilatation.

2. Collection of bile

A small flexible rubber tube about 7 cm. long is passed into the bile fistula, and should fit accurately. This tube passes through the short stem of a glass funnel, and is fixed firmly in it. This serves two purposes —first, the funnel catches a little mucus which oozes from the edge of the fistula, and prevents its mixture with the bile secretion; second, it will show any escape of bile about the tube which never occurs in suitable fistulae, and serves to hold the tube firmly in place in the fistula. The glass funnel is held tight against the abdomen and fistula by a binder reinforced by metal about the funnel. The binder is held accurately in position by adhesive plaster over the dog's back, and a small rubber bag is fixed to the end of the glass funnel stem to catch all the bile. Wide cloth and webbing strips are passed under the thorax and forelegs to prevent the dog from lying down on the rubber bag and spoiling the collection. The dogs stand or sit on their haunches, and doze quietly a good part of the period of collection.

3. Bile pigment estimation

One cubic centimeter of the bile to be analyzed is added to 49 cc. of the following solution (ethyl alcohol 95 per cent, 100 cc., nitric acid concentrated, 0.4 cc., and hydrochloric acid concentrated, 2 cc.) and mixed in a volumetric flask. The flask is shaken thoroughly, corked, and allowed to stand at room temperature about eighteen hours, when the readings are made. This solution turns bluish green, and reaches its maximum color in twelve to eighteen hours, and holds its intensity for twenty-four to forty-eight hours or longer. The solution is filtered through paper, and read in a colorimeter (Autenrieth-Königsberger as modified by Rowntree and Geraghty). The method is very simple and accurate to 0.01 mgm. of bilirubin.

The bile pigments in the serum or urine are estimated as follows. The fluid is made alkaline with a saturated solution of sodium carbonate and mixed with a 10 per cent solution of calcium chloride giving a voluminous precipitate containing the bile pigments. The precipitate is thrown down and washed repeatedly with distilled water by use of the centrifuge. The precipitate is finally dissolved in a measured amount of the nitro-hydrochloric acid alcohol solution, and allowed to stand at room temperature over night. The pigments are then estimated by the colorimeter.

For this work it is very desirable to have a permanent standard wedge for the colorimeter, and after many trials the best result was obtained as follows. A normal solution of very pure copper sulfate is treated with a few drops of a dilute watery solution of India ink. This suspension is not permanent unless fixed in some way. This is accomlished by a solution of agar-agar and gelatin which must be cleared with great care. Equal parts of the gelatin-agar solution and normal copper sulfate solution are combined while still warm, and poured into the standard wedge. The mixture of copper sulfate, ink, and gelatin-agar when it cools is permanent, and the wedge may be sealed with vaseline. Our standard wedge had been standardized against (a) pure bilirubin obtained from human gall stones, (b) bilirubin C. P., Kahlbaum, source of bile unknown, and (c) pure crystalline dog bilirubin. A table has been constructed so that knowing the colorimeter reading and the amount of bile in cubic centimeters, the bile pigment can be read off directly.

Table I (Dog 16–6) shows the output of bile and bile pigments by a dog in good condition on mixed diet plus cooked liver. The bile

secretion varies in six hours from a minimum of 42 cc. to a maximum of 69 cc. and the bile pigments from 29 mgm. to 42 mgm. The two hour periods are seen to vary greatly, but there is no relation to food which is always given at the end of the first two hours. Bile is excluded from the intestine and stercobilin is absent. There are only traces of bile pigment in the urine. This bull dog was very fat when operated upon, and the initial loss of weight during the first month is in part due to this fact. It is noted that bile fistula dogs in the best possible

TABLE I

Normal dog—mixed diet and liver

DOG 16-6*	BILE								URINE BILE PIGMENTS TOTAL, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 16	17	16	19	52	7.4	9.8	16.6	33.8	trace	36.5	R.B.C. 5,632,000: Hb. 87 per cent: W.B.C. 7,600.
September 17	24	20	16	60	11.7	10.5	6.7	28.9	0	36.3	Stools clay colored; no stercobilin.
September 18				62				32.1	0	36.0	
September 20	16	18	21	55	11.2	15.8	9.7	36.7	0	35.5	
September 21	24	24	18	66	15.4	14.0	13.0	42.4	trace	36.0	
September 22	23	21	18	62	14.1	13.1	13.4	40.6	0	35.5	
September 23	20	22	27	69	12.5	11.6	12.2	36.3	+	35.8	Stools clay colored; no stercobilin.
September 24	13	25	19	57	6.4	12.7	11.4	30.5	+	35.5	
September 25				42				33.0	trace	34.5	
September 27	23	18	14	55	13.5	8.3	8.1	29.9	+	34.0	September 30. Hb. 98 per cent.
Average				58				34.4			

* Bile fistula operation September 2, 1915. Usual mixed diet plus 150 grams boiled sheep liver with morning meal.

condition will carry but very little subcutaneous fat. If the dog is lean and muscular at time of operation, it will lose only a little weight, but if very fat, the dog will lose several pounds after operation in spite of any care and apparent good health and appetite.

Table II (Dog 16–6) shows the same dog as Table I two months later when in apparently excellent condition. The dog was fed a mixed diet plus cooked liver and fresh dog bile. It is to be noted that the bile volume is much greater than in Table I (average per six hours—58 cc.) as compared with the average of 96 cc. We believe this chola-

gogue action is wholly due to the fresh bile feeding. On the other hand the bile pigments are *lower* (average 28.6 mgm. per six hours) as compared with Table I (average 34.4 mgm. per six hours). This drop in bile pigment output we do not believe is due to loss in weight, as the dog seemed quite normal and had a good appetite. We will discuss this point more in detail later.

The animal seemed normal in all respects until December 5, when she vomited some food. The next day she developed muscular tremors and convulsions shortly followed by death. The intoxications which

TABLE II

Normal dog—mixed diet, liver and bile

DOG 16-6*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
November 26.....	34	36	29	99	10.8	9.6	9.8	30.2	trace	30.8	
November 27.....				102				27.6	trace	30.8	Stools clay colored; no stercobilin.
November 29.....	35	35	29	99	7.9	8.6	8.5	25.0	trace	31.0	
November 30.....	35	26	27	88	10.1	10.5	9,5	30.1	0	30.8	
December 1.....	30	31	33	94	12.2	8.9	9.0	30.1	0	30.3	Hemoglobin 108 per cent; beef heart diet.
Average.....				96				28.6			

* Bile fistula operation September 2, 1915. Death, intoxication December 6, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal and 40 cc. fresh dog bile mixed with morning meal.

develop in consequence of long standing bile fistulas are of great interest but cannot be discussed at this time.

Autopsy in general is negative. Kidneys and other organs are normal. Liver is practically normal; no increase in fat. There is slight increase in the brownish color of the liver cells. The bile passages are all clean and pale throughout, even at the lower part of the fistula in contact with the drainage tube when it is in place. The common duct and the hepatic ducts are slightly dilated and thickened. Site of section of common duct is obliterated by dense scar tissue. The duodenal papilla is normal, and contains only mucus and no bile. Bile is completely excluded from the intestine.

Table III (Dog 15–22) shows the initial loss of weight following a bile fistula from 30.8 pounds to 24.5 pounds due to the fact that the dog was quite fat when operated upon. The bile flow varies from 66 cc. to 97 cc. per six hours (average 76 cc.), and the bile pigments from 20 mgm. to 32 mgm. per six hours (average 25.5 mgm.), but the maxima and the minima for bile flow and bile pigments do not coincide in any way. Bile pigments are constantly present in small amounts in the urine. The usual hourly variations in secretion are noted. Stercobilin is absent, and bile presumably absent from the intestine, which is to be contrasted with Table IV in the same dog.

TABLE III

Normal dog—mixed diet and liver

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									mgm.	*pounds*	
April 21	33	30	34	97	9.6	9.5	7.8	26.9	0.25	27.3	April 20, R.B.C. 5,952,000: Hb. 94 per cent: W.B.C. 8,000.
April 22	25	24	26	75	7.1	9.1	11.9	28.1	+	27.5	
April 23	25	26	26	77	8.8	11.1	11.9	31.8	+	27.0	Stools clay colored; no stercobilin.
April 26	27	21	23	71	7.2	8.0	10.1	25.3	0.3	25.5	R.B.C. 5,824,000; Hb. 96 per cent.
April 27	15	26	25	66	3.6	8.3	7.9	19.8	0.2	25.0	
April 28	21	22	15	68	6.5	7.4	7.0	20.9	0.3	24.5	
Average				76				25.5			

* Bile fistula operation April 2, 1915, weight 30.8 pounds. Usual mixed diet plus 100 grams boiled sheep liver with morning meal.

Table IV (Dog 15–22) is of considerable interest because the dog's stools now contain some stercobilin. This period is about four months after Table III observations of April. In May it was noted that the dog had gained weight up to his original weight of 30.8 pounds, and was in unusual condition for a bile fistula dog on a liver and mixed diet. Stercobilin was found in the feces constantly from this time on, and we were forced to the conclusion that by means of a small fistulous tract (as observed previously in the dogs at autopsy) a small amount

of bile escaped into the duodenum when the pressure in the common duct was elevated.

From great numbers of observations on this dog and others with no bile escaping into the duodenum at any time, we are convinced that the collections from this dog, 15–22, represent his total output during the periods of six hours, we know that such fistulous tracts as this dog must have are very small and tortuous, and permit of only small amount of bile escaping even under high pressure. His output (Table III), before this fistulous tract into the duodenum was established, is practi-

TABLE IV

Normal dog—mixed diet and liver—bile in intestine

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs	5-6 hrs.	Total 6 hrs.			
										pounds	
August 17	18	16	18	52	10.2	7.9	8.6	26.7	0	33.3	Hemoglobin 129 per cent
August 18	14	11	18	43	9.1	9.0	9.7	27.8	0	33.3	
August 19	17	14	16	47	9.2	8.5	8.8	26.5	0	33.3	
August 20	16	17	15	48	9.4	7.8	8.6	25.8	0	33.3	Stools contain stercobilin
September 15	27	17	25	69	8.3	6.3	6.8	21.4	0	33.	
September 16	23	17	28	68	9.1	8.2	10.7	28.0	0	32.8	
September 17	18	20	26	64	8.1	9.7	11.1	28.9	0	33	R.B.C. 7,848,000: Hb. 125 per cent: W.B.C. 9,600
September 18				56				26.7	0	33	
Average				56				26.5			

* Bile fistula operation April 2, 1915. Usual mixed diet plus 200 grams cooked sheep liver with morning meal.

cally identical with that in Table IV, the after period. His output of bile compares exactly with that of similar dogs with complete fistulae, shows the same fluctuations and the same average output. When the dog is put up in harness with a tube draining his gall bladder, it seems almost certain that all the bile escapes through this tube as the flow takes place by gravity. When the dog is curled up in his cage with his bile fistula partially closed and compressed by his posture, much bile escapes into the cage, and a small amount escapes into the duodenum along the path of the resected and ligated common duct. This bile is sufficient to maintain the dog in normal condition.

Table V (Dog 15–22) resembles in general Table IV, and shows the same constantly high hemoglobin curve. There is a little loss of weight, but the dog is in perfect condition. This table covers a period of five days *following* a period of four days of sexual intercourse and excitement, during which period of over excitement the bile output was double normal. We have more data on this point which we will report later. These data are given to explain in part at least the abnormally high initial curve of pigment excretion in this dog with the progressive fall during the week (Table V).

TABLE V

Normal dog—mixed diet

DOG 15-22*	BILE								URINE BILE PIGMENT TOTAL — SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1915										*pounds*	
November 26....	13	18	19	50	12.5	15	14.5	42.0	0	31.0	November 11, hemoglobin 121 per cent.
November 27.....				39				36.8	0	31.3	Stools contain stercobilin
November 29.....	19	22	20	61	7.0	16.5	12.9	36.4	0	31.5	
November 30.....	23	23	23	69	6.2	7.4	8.8	22.4	trace	31.3	Hemoglobin 124 per cent
December 1.....	21	22	25	68	9.4	10.1	10.7	30.2	0	31.3	
Average.....				57				33.6			

* Bile fistula operation April 2, 1915. Mixed diet of meat, bones, bread, and no liver.

Table VI (Dog 16–5) is a good example of the fluctuation of the bile pigment curve which may be found following a bile fistula operation associated with icterus of a mild but definite degree. For some reason drainage was not good after the operation, and for many daysafter normal drainage was established we see the wide fluctuation in output from a minimum of 18 mgm. to a maximum of 58 mgm. The weight remains constant, and the dog is active and hungry. Any procedure taken up during any such period could give no information, and would only lead to confusion. We believe the icterus is in part responsible for these great fluctuations in bile pigment excretion. Gradually the curve of pigment excretion becomes more uniform as seen in the next Table VII.

Table VII (Dog 16–5) again shows the cholagogue action of bile given with the food (compare Table VI), and gradually the bile pigment excretion becomes fairly uniform.

One notes on two days (October 1 and 5) that the second period of collection shows a very small excretion. This is not due to any error in collection, as the dog was under constant observation, and we are not prepared to give a satisfactory explanation. It has been noted

TABLE VI

Post-operative icterus—mixed diet and liver

DOG 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 15.....	22	15	15	52	17.8	15.3	16.9	50.0	++++	30.5	R.B.C. 5, 472,000: Hb. 89 per cent: W.B.C. 8,200: definite jaundice.
September 16.....	20	15	20	55	21.7	19.7	17.2	58.6	++++	31.0	Stools contain no stercobilin.
September 17.....	22	15	24	61	13.9	12.2	21.1	47.2	+++	31.3	
September 18....				47				34.9	++	31.5	
September 20.....	19	9	11	39	22.5	16.4	14.5	53.4	+	30.5	
September 21.....	24	8	21	53	12.7	4.2	14.2	31.1	+	29.5	Slight jaundice
September 22.....	13	8	14	35	4.3	4.0	9.5	17.8	+	29.5	
September 23.....	7	17	24	48	2.5	7.8	10.8	21.1	++	29.0	Stools contain no stercobilin.
September 24.....	20	17	33	70	3.1	5.0	11.9	20.0	++	29.0	
September 25.....				46				12.4	++	29.3	Slight jaundice.
September 27.....	14	21	19	54	7.0	10.6	9.2	26.8	++	29.8	
September 28.....	23	15	16	54	6.5	6.1	6.3	18.9	++	30.0	
Average.....				53				32.7			

* Bile fistula operation September 1, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

that soon after operation (two weeks) the introduction of the rubber tube may be followed by an hour or more of almost complete cessation of the bile flow. This may be due to a nervous reflex resulting from the slight pain caused by the catheter in the recent fistula or due to the excitement of the novel surroundings and an unfamiliar procedure. We may be able to give a satisfactory explanation as more observations accumulate, but at present we believe such periods of inhibition of flow may be due in part to some nervous reflex.

TABLE VII

Slight icterus—mixed diet and fresh bile

DOG 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 29	27	17	23	67	8.3	4.0	9.5	21.8	++	30.3	Stools contain no stercobilin.
September 30	15	24	20	59	6.2	5.5	7.4	19.1	++	30.5	
October 1	34	8	36	78	10.7	2.9	11.3	24.9	++	30.5	Slight jaundice
October 2				62				29.3	++	30.5	
October 4	27	16	20	63	9.1	6.5	8.8	24.4	++	30.8	
October 5	31	10	25	66	12.4	4.3	12.4	29.1	++	31.0	
October 6	26	21	25	72	9.8	10.3	10.1	30.2	++	30.8	October 22—R.B.C. 6,360,000: Hb. 93 per cent: W.B.C. 13,400.
Average				67				25.5			

* Bile fistula operation September 1, 1915. Mixed diet plus 30 cc. fresh dog bile with morning meal and 60 cc. fresh dog bile with evening meal.

TABLE VIII

Poor condition—duodenal ulcer

DOG 15-16*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									mgm.	*pounds*	
April 14	55	27	44	126	17.3	7.7	10 9	35.9	0	42	April 5, R.B.C. 5,776,000: Hb. 90 per cent: W.B.C. 7,200.
April 15	41	42	24	107	9.2	10.3	6.6	26.1	0	41.5	Stools contain no stercobilin.
April 16	35	53	19	107	8.5	15.5	6.8	30.8	0	41.0	
April 19	22	27	30	79	18	23.1	28.5	69.6	0.7	39.5	Slight jaundice.
April 20	25	27	23	75	14.6	16.9	19.0	50.5	0.5	39.3	R.B.C. 5,408,000: W.B.C. 7,000. Stools contain no stercobilin.
Average				99				42.6			

* Bile fistula operation March 24, 1915. Death April 28. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

Table VIII (Dog 15–16) shows a good example of a dog which lost ground steadily after the operation in spite of careful feeding and every attention. His bile output is fairly constant, but the bile pigment secretion varies from 26 to 70 mgm. per six hours. He developed a slight grade of icterus and lost all appetite. He was sacrificed because of poor general condition.

Autopsy gave the following information. Liver seemed normal in gross, but under the microscope showed a little fatty degeneration. Bile passages all normal, and bile completely excluded from duodenum. Large duodenal ulcer (1.5 by 1 cm.) about 3 cm. below pylorus extending deep into muscular coats gives no evidence of hemorrhage. Prostate is huge. Microscope shows the common cyst adenoma of the gland. The dog was quite old. The old age and the duodenal ulcer may be in part responsible for the rapid failure of this dog after the bile fistula operation. However, he showed some signs of the characteristic intoxication which so frequently carries off the animals.

DISCUSSION

A study of the above tables will emphasize the point which Stadelmann insists upon with so much justice, namely, the normal flow of bile from a bile fistula dog is subject to wide fluctuations, which cannot at present be explained. This includes fluctuation in the total output and the bile pigment secretion, but it is to be noted that these maxima do not coincide. A low output of bile may contain a high total bile pigment content and vice versa. Stadelmann points out the fact that bile pigment and bile salt secretion have no relation, and he argues from this that the function of the liver cell is double and quite independent in these two respects. With this point in mind, it is obvious that one must be very careful in analysis of any observations on bile secretion. Experiments must be repeated again and again, and a seemingly unnecessary number of control observations must be recorded before and after the actual experiment. It is seen in the above tables that with care and proper food a dog may have a fairly long period of relatively uniform secretion. Such periods are most favorable for experimental work.

We realize that criticism may be offered against our six hour period of bile collection. Many of the recorded experiments of other workers show twenty-four hour collections or even periods of several days during which time the dog is suspended comfortably in a sling. Others

use ten or twelve hour periods for several consecutive days. After some observations, it seemed best in the long run to make shorter daily collections over many weeks or months, obtaining the bile every day during the same hours, the dog having a constant daily routine. The dog can live a pretty normal existence, and, most important of all, can maintain a pretty constant bodily condition for a long period of time. Such bile collections perhaps represent more nearly the normal flow as it occurs in a normal dog under laboratory conditions.

It will be seen after a careful study of the above tables that these bile fistula dogs on the mixed diet have a pretty uniform *average* excretion of bile pigments—about 1 mgm. per pound body weight per six hours. Our animals are even more constant in this respect than those studied by Stadelmann and his co-workers, but our average is practically identical with their published reports. This indicates that the method used by Stadelmann (spectrophotometric) gives the same general results as our method. The figures given by Brugsch, Kawashima and Yoshimoto are about three times as high as those just reviewed, and can scarcely be accepted as correct.

SUMMARY

Our experiences with bile fistula dogs indicate that bile is essential for the life of the animal on a mixed diet of meat, bones, and bread. If bile is *wholly* excluded from the intestinal tract, the dog loses ground steadily, shows intestinal disorders accompanied by blood in the feces, and usually within a month dies with peculiar symptoms of intoxication.

Fresh pig's bile given by stomach tube and dried ox bile given in capsules will sometimes improve the condition but not to any notable degree.

Fresh dog's bile mixed with the food will sometimes give good results if the dog will eat the mixture. Given by stomach tube the results are not favorable.

Cooked liver added to a mixed diet usually keeps the dog in good healthy condition for a long period of time. At present we are not prepared to explain this observation, but the fact may have some clinical application.

Under very uniform conditions the bile pigment excretion may form a pretty uniform curve, and experimental variations under such circumstances will have some value. The usual average bile pigment excretion amounts to one miligram per pound body weight per six hours,

but there are some individual variations and considerable daily and hourly variation.

When a dog is not in good condition and perhaps is suffering from icterus or cachexia or both, we may see very great fluctuations in the bile pigment excretion curve. Experimental observations under such conditions are worse than useless, and can lead to no conclusions of value.

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BILE PIGMENT METABOLISM

II. Bile Pigment Output Influenced by Diet

G. H. WHIPPLE and C. W. HOOPER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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The preceding paper shows the normal bile pigment secretion in dogs with permanent bile fistulae. The normal fluctuations are obvious, and must be taken into consideration in the analysis of any experiments. This paper gives observations which make it clear that the curve of bile pigment secretion can be depressed below normal by a meat diet, and can be raised much above normal by a diet rich in carbohydrates.

It seems that this observation must dispose of the commonly accepted belief concerning the origin of bile pigments; namely, that they can be formed only by the breaking down of red blood cells. Can one assume that a carbohydrate diet will cause the dissolution of a small army of red blood cells to explain the fact that the output of bile pigment may be almost doubled in a sharp transition from a meat diet to a diet rich in carbohydrates? This seems improbable to say the least.

Methods and operative procedures, care of animals and collection of bile have all been described in detail in the preceding paper. We can not give all our experimental data on this diet question, but there is complete agreement in the fundamental observation that a dog will show a low bile pigment output on a meat diet and a definite increase (sometimes 100 per cent increase) on a diet rich in carbohydrates. The two following experiments are given in considerable detail, because the two dogs were under observation for a long period of time, in perfect health, showed a constant hemoglobin curve, and only traces of bile pigment in the urine at times. The observations on a single large dose of carbohydrate can be multiplied indefinitely, but they confirm the more difficult prolonged diet period experiments extending over weeks.

The Tables A, B, and C show that sugar by mouth will cause an increase in bile pigment output in a dog on a meat diet. There is a slight decrease in total bile flow during this period.

TABLE A

Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Lean beef diet plus 200 grams cooked liver
										pounds	
October 21	24	23	26		10.0	1.4	13.2		0	32.0	
October 22	20	13*	12	22	9.2	14.3*	17.7	17.4	0	31.5	R. B. C. 7; 640,000; Hb. 123 per cent: W. B. C. 8600.
October 23	24				13.7				0	31.8	Stools contain stercobilin.
October 25	22	22	23		10.9	9.7	10.9		trace	32.0	
October 26	17	18	16		8.0	9.3	7.9		0	31.8	

* Given 80 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

TABLE B

Dextrose feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Lean beef diet plus 200 grams cooked liver
										pounds	
June 7	24	25	22		4.3	4.5	5.3		trace	31.5	Stools contain stercobilin
June 8	19	18*	11	14	4.6	6.7*	7.6	9.1	trace	31.5	
June 9	11	11	12		4.5	5.2	7.6		trace	30.5	
June 10	23	24	26		4.1	4.8	4.7		trace	30.8	

* Given 200 grams dextrose in 400 cc. water by stomach tube at beginning of third hour.

TABLE C

Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Lean beef diet plus 200 grams cooked liver
										pounds	
October 14	22	24	22		5.9	10.0	11.1		0	32.5	
October 15	28	14*	15	16	5.6	8.5*	14.2	20.9	0	33.0	
October 16	22				11.9				0	32.8	
October 18	31	26	29		10.3	8.0	8.5		0	32.0	Stools contain stercobilin
October 19	22	24	27		9.2	8.5	10.1		0	32.0	

* Given 60 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

Tables D and E show that dextrose given intravenously will also cause a rise in the bile pigment curve of excretion, one experiment on a mixed diet and the second on beef heart diet. These experiments

TABLE D

Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Mixed diet plus 200 grams cooked liver
										pounds	
May 18	19	21	18		6.8	7.1	7.0		trace	30.0	May 15, R.B.C. 5,888,000 Hb. 93 per cent.
May 19	19	23*	34	27	3.9	6.2*	12.1	10.0	trace	30.5	
May 20	18	20	21		3.6	5.4	7.1		+	31.0	Stools contain stercobilin. May 22, R.B.C. 5,696,000: Hb. 91 per cent.
May 21	24	24	24		5.4	7.0	6.9		trace	31.0	

* 50 grams dextrose in 1000 cc. 0.7 per cent salt solution given intravenously at beginning of third hour.

TABLE E

Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Beef heart diet
										pounds	
December 13	32	23	27		7.2	6.7	8.5		0	31	December 11, hemoglobin 131 per cent.
December 14	33	32	23		8.0	9.4	8.6		0	31.5	Stools contain stercobilin.
December 15	31	29*	16	15	7.5	11.5*	10.5	8.1	0	31.8	
December 16	23	22	23		14.1	14.5	12.2		0	31.0	
December 17	26				12.7				0	30.5	

* 600 cc. 6 per cent dextrose given intravenously at the beginning of the third hour.

show little decrease in bile flow, especially in Table D, where the larger amount of fluid (1000 cc.) was given intravenously. Such variations, however, come within physiological limits, and no importance is to be attached to them.

Table F shows in a convincing way that a meat diet gives a much lower output than a diet rich in carbohydrates. The meat diet period of six days shows an average output of 29.1 mgm. bile pigment, which is not very low, as can be seen in the after-period of eleven days with an average of 25.6 mgm. (Table H). This same dog at other times on a meat diet has gone as low as 16 mgm. bile pigment output average of six days.

TABLE F

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Boiled lean meat diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1915										*pounds*	
October 25	22	22	23	67	10.9	9.7	10.9	31.5	trace	32	October 22, R.B.C. 7,640,000 Hb. 123 per cent.
October 26	17	18	16	51	8.0	9.3	7.9	25.2	0	31.8	
October 27	20	18	19	67	6.1	9.8	8.1	24.0	trace	31.8	
October 28	18	17	21	56	9.0	9.6	12.0	30.6	trace	31.5	
October 29	20	21	26	67	9.5	11.3	11.1	31.9	trace	31.8	
October 30				56				31.5	+	31.0	Stools contain stercobilin.
Average				59				29.1			
Change from lean meat diet to bread, milk and bones											
November 1	15	16	18	49	13.5	15.1	16.	44.6	trace	31.5	Stools contain stercobilin.
November 3	16	18	20	54	14.4	17.1	16.7	48.2	trace	31.8	October 25, Hb. 123 per cent.
November 4	17	23	20	60	16.0	19.1	16.3	51.4	trace	31.8	
November 5	15	20	19	54	13.5	13.5	13.3	40.3	trace	32.0	
November 6				45				41.5	trace	31.8	
Average				52				45.2			

The sharp transition to the diet of bread, milk and bones gives a great rise to an average of 45.2 mgm. bile pigment, an increase of over 50 per cent bile pigment elimination. There is a trifling decrease in average bile flow from 59 cc. to 52 cc.

Table G shows that a continuation of the bread, milk, and bone diet does not maintain the bile pigment output at the maximum of 45.2 mgm. of the previous week, but the average is 39.7 mgm. bile pigment. This same fact is noted in another dog (Table K), and there is

a tendency for the high bile pigment curve of a carbohydrate diet to approach the mean curve of a mixed diet. Also there is the same tendency for the low bile pigment curve of a meat diet to approach the mean curve of a mixed diet. This applies particularly to dogs kept for several weeks on a meat diet or a carbohydrate diet. The meat diet dog may show periods of rise in bile pigment output close to the mean curve of a

TABLE G

Bile pigment secretion on carbohydrate diet and fat

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			Diet of bread, milk, and bones
										pounds	
November 8.....	17	20	24	61	11.9	13.6	12.7	38.2	0	31.3	
November 9.....	19	23	21	63	13.3	11.2	12.8	40.3	0	31.3	
November 10.....	21	21	25	72	8.0	9.2	12.4	29.6	trace	31.3	
November 11.....	28	26	25	79	11.1	11.4	10.5	33.0	trace	31.5	Hemoglobin 121 per cent
November 12.....	16	23	22	61	12.6	13.2	12.6	38.4	trace	31.3	
November 13.....				55				42.5	trace	31.3	
November 15.....	14	23	21	58	14.5	13.2	14.4	42.1	0	31.0	
November 16.....	18	23	20	61	15.0	18.2	12.6	45.8	trace	31.5	
November 17.....	20	19	23	62	16.7	15.8	15.2	47.7	trace	31.3	
Average.....				64				39.7			
Same diet plus 100 cc. cotton seed oil with morning and evening feeding											
November 18.....	13	18	19	50	15.2	16.6	16.2	48.0	0	31.5	
November 19.....	24	23	19	66	10.8	11.1	9.6	31.5	0	31.8	
November 20.....				65				27.8	0	31.5	November 30, hemoglobin 124 per cent.
Average.....				60				35.7			

mixed diet. The diet rich in carbohydrate may give a very high initial curve (perhaps double normal), which is apt to fall during succeeding weeks, but always remains somewhat above the mean curve.

Cotton seed oil fed with this bread, milk, bone diet is associated with a slight drop in bile pigment elimination, but another dog (Table K) gives negative results. We hope to do much more work with various fats and lipoids.

Table H shows the after-period on a beef heart diet with an average output of 25.6 mgm. bile pigment. The flow of bile is somewhat increased on this diet from 60 cc. to 83 cc. per six hours.

The importance of these observations (Tables F, G, H) lies in part in the fact that this dog was under constant observation with daily collections of bile for a period of about eight weeks in perfect health with uniform hemoglobin curve. The deduction to be drawn from changes in diet under such uniform conditions will be of value, and the sequence of events is not to be lost sight of: (1) Meat diet and low

TABLE H

Bile pigment secretion on beef heart diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
December 2	39	27	28	94	7.8	10.3	9.9	28.0	trace	32.0	November 30, hemoglobin 124 per cent.
December 3	25	26	28	79	8.3	8.2	8.1	24.6	0	32.2	
December 4				79				24.9	0	31.3	
December 6	24	21	22	67	9.5	9.9	7.7	27.1	0	31.0	
December 7	27	28	31	86	8.3	10.1	12.3	30.7	0	31.5	Stools contain stercobilin
December 8	34	33	24	91	5.4	10.4	8.3	24.1	0	31.8	
December 9	38	28	26	92	5.1	6.7	7.6	19.4	0	31.8	
December 10	33	35	24	92	7.3	10.9	8.5	26.7	0	31.8	
December 11				64				28.2	0	31.5	Hemoglobin 131 per cent
December 13	32	23	27	82	7.2	6.7	8.5	22.4	0	31.0	
December 14	33	32	23	88	8.0	9.4	8.6	26.0	0	31.5	Stools contain stercoblin.
Average				83				25.6			

bile pigment elimination: (2) bread, milk, bone diet and very high bile pigment curve: (3) second period of bread, milk, bone diet and constant high pigment curve; (4) same diet with oil shows slight fall in bile pigment curve: (5) end period of beef heart diet with low bile pigment elimination (see table M).

Table J confirms the observations in Table F, but the change here is not so striking. On a meat diet the dog put out 27.7 mgm. bile pigment, and on a bread, milk, bone diet eliminated 37.5 mgm. bile pigment. It is to be noted that this dog was losing weight during this

period, but seemed in good health, and the hemoglobin curve was uniform.

Table K shows a slight fall during the second week of carbohydrate feeding, but it remains above the meat diet period, and shows no depression as the result of adding cotton seed oil to the same diet.

TABLE J

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			Boiled lean meat diet plus 200 grams boiled liver
										pounds	
October 25	30	27	22	79	10.8	10.3	9.7	30.2	0	34.5	October 22, R.B.C. 7,640,000; Hb. 105 per cent; W. B. C. 8,600.
October 26	26	25	27	78	7.6	7.7	8.9	24.2	0	34.0	
October 27	25	24	23	72	7.3	7.9	9.6	24.8	0	33.8	Stools contain no stercobilin.
October 28	24	20	21	62	10.0	8.3	10.6	28.9	trace	33.3	
October 29	25	23	16	64	10.1	10.4	9.4	29.9	0	33.0	
October 30				54				27.8	0	32.5	Hemoglobin 103 per cent
Average				68				27.7			
Change from lean meat diet to bread, milk and bones											
November 1	16	18	20	54	10.8	13.8	11.9	36.5	0	31.5	October 30, hemoglobin 103 per cent.
November 3	17	18	20	55	13.4	11.3	15.0	39.7	0	31.5	
November 4	22	20	22	64	12.9	13.2	13.1	39.2	0	31.0	
November 5				54				37.8	trace	30.8	
November 6				66				34.1	trace	30.8	Stool contain no stercobilin.
Average				59				37.5			

The after-period (Table L) is unsatisfactory because rather too short as a result of the death of the dog. She was apparently in perfect health December 4, and it is proper to include this reading in the table. The next day she refused food, and vomited once, but did not appear sick. On December 6 she died with peculiar symptoms of intoxication, and the autopsy abstract is given in the preceding paper (following Table II—Dog 16-6).

TABLE K

Bile pigment secretion on carbohydrate diet and fat

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			Bread, milk and bone diet plus 200 grams boiled liver
										pounds	
November 15	23	31	29	83	9.3	10.3	10.3	29.9	trace	31.5	October 30, hemoglobin 103 per cent.
November 16	30	31	29	90	10.0	11.7	10.3	32.0	trace	32.0	
November 17	22	28	35	85	9.7	13.0	8.6	31.3	trace	32.3	Stools contain no stercobilin.
Average				86				31.1			
Same diet plus 100 cc. cotton seed oil with morning and evening feeding											
November 18	28	27	30	85	10.5	8.4	8.8	27.7	trace	32.5	
November 19	35	33	35	103	14.8	8.8	8.7	32.3	trace	32.5	
November 20				112				30.2	0	32.5	
November 22	18	28	34	80	11.9	12.4	12.1	36.4	trace	31.0	
November 23	29	22	34	85	10.3	10.0	9.8	30.1	trace	31.8	
November 24	35	23	31	89	11.0	12.2	10.5	33.7	trace	31.3	December 1, hemoglobin 108 per cent.
Average				92				31.7			

TABLE L

Bile pigment secretion on beef heart diet

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in millimeters						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			Boiled beef heart diet plus 200 grams boiled liver
										pounds	
December 1	30	31	34	95	12.2	9.0	9.0	30.2	0	30.3	Hemoglobin 108 per cent
December 2	35	39	30	104	7.8	9.9	8.1	25.8	0	30.0	
December 3	36	35	31	102	7.3	7.8	6.9	22.0	0	30.8	
December 4				112				17.6	0	31.3	
Average				103				23.9			

TABLE M

Average readings from above tables

DOG	LEAN MEAT DIET		BREAD, MILK, BONE		BREAD, MILK, BONE DIET PLUS OIL		BEEF HEART DIET		REMARKS
	Bile	Bile pigment	Bile	Bile pigment	Bile	Bile pigment	Bile pigment	Bile pigment	
	cc.		cc.		cc.		cc.		
Dog 15-22	59	29.1	52	45.2					
Average per 6 hours			64	39.7	60	35.7	83	25.6	Weight, 32 pounds
Dog 16-6	68	27.7	59	37.5					
Average per six hours			86	31.1	92	31.7	103	23.9	Weight, 31 pounds

This table gives the average readings of bile and bile pigment output during the various diet periods. They are arranged in the same sequence in which the experiments were performed. For study of the details one must refer to the other tables which are of greater interest.

DISCUSSION

From the above tabulated experiments it is clear that a diet rich in carbohydrates or sugar by mouth or dextrose intravenously will increase the secretion of bile pigments. We believe the data are sufficient to establish this as a fact, but how may we explain this increase in bile pigments following the administration of a carbohydrate? There are numerous possibilities which must be tried out by various experimental procedures, and we will merely mention a few of them.

Sugar feeding at once suggests a storing of glycogen in the liver, and its deposition in the liver cell may accelerate the metabolic activity of the cell or stimulate it to produce greater amounts of bile pigments than under normal conditions.

We must recognize, too, that a meat diet tends to depress the bile pigment secretion below the usual level of a mixed diet. Any explanation suggested to explain the carbohydrate stimulus must also explain the protein diet depression of bile pigment output. It is possible that a meat diet in dogs represents a normal condition, and that the low curve of pigment excretion on the meat diet is the true normal excretion. The rise on a simple mixed diet of bread and meat may then be explained by the addition of the carbohydrate and the maximum output on the bread, milk, bone diet as due to a great increase in the

carbohydrate portion of the diet. It will be of considerable interest to observe the bile pigment output on a pure carbohydrate diet. This is a difficult type of experiment for a bile fistula dog, but it may give results of considerable value when carried to a successful termination.

Here it may be mentioned that we have pretty good evidence that bile or blood feeding do not greatly increase the bile pigment output. We hope to report detailed work on this important point in the near future.

When it is suspected that the liver can form bile pigments out of various materials other than hemoglobin, which is so closely related chemically to bile pigment, one first thinks of substances rich in the pyrrhole nucleus. Bile pigment or blood feeding should give a great increase in bile pigment output, which is not the fact. One must go back further in the development of the body pigments and ask; where does the hemoglobin come from? A prompt answer is made that hemoglobin is formed in the red cells in the bone marrow. But it is at least possible that these cells may merely put the finishing touches on this complex substance, which may be built up in great measure in some other tissue. In other words, there may be a prehemoglobin substance manufactured somewhere in the body, perhaps in the liver, which may be fixed by the bone marrow cells, and appear as finished hemoglobin. If it can be established that the liver cells form any such substance, a long step will have been made toward the solution of this complex question of pigment metabolism.

SUMMARY

A large dose of sugar by mouth will give a constant reaction in a healthy dog with a bile fistula. It will cause a definite increase in bile pigment excretion over a period of several hours.

The same rise in the curve of bile pigment elimination follows intravenous injection of dextrose.

A mixed diet in a healthy bile fistula dog is associated with a fairly constant *mean* bile pigment elimination.

A change to a meat diet will give a depression of this average bile pigment elimination.

A change to a diet rich in carbohydrates will give a sharp rise in bile pigment output—often 30 to 100 per cent increase. Such modifications of bile pigment elimination may be carried on indefinitely with a healthy animal.

We believe established the fact that carbohydrates stimulate the excretion of bile pigments in bile fistula dogs, but a convincing explanation of this phenomenon we can not bring forward at this time. More work is required.

It seems, howevre, that these facts must overthrow the long accepted theory that bile pigment is formed only as a result of the disintegration of red blood cells.

It is at least possible that the liver has some constructive ability in pigment formation which can be modified by diet. It is also possible that the liver may be concerned in building up other body pigments than bilirubin—for example, hemoglobin.

THE INFLUENCE OF HYPOTENSIVE GLAND EXTRACTS ON VASOMOTOR IRRITABILITY

ARTHUR F. BEIFELD, HOMER WHEELON AND C. R. LOVELLETTE

From the Laboratory of Physiology of the Northwestern University Medical School

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The observations herein recorded were obtained in the course of an investigation of the relation of pancreas extracts to vasomotor conditions. The original aim of our work was to determine whether or not pancreas extracts contain a specific hormone which could account for the favorable results which one of us (B.) has obtained in the clinical use of such material in arteriosclerosis, particularly in angina pectoris (1). Though this problem was not satisfactorily solved, certain observations made during the study have seemed worthy of record.

The technique employed was essentially that described in other reports from this laboratory (2). Dogs, under ether anesthesia, were used in all cases as experimental animals. These were first given standard injections of epinephrin and nicotin—of the former 1 cc. of a 1:50,000 solution, and of the latter 1 cc. of a 1: 2,000–4,000 solution—and the vasomotor responses noted by means of a mercury manometer and float. These reactions served as an index, respectively, of the condition of the peripheral musculature and of the vasomotor centers—a conclusion warranted by the work of Elliott (3), and of Langley and Dickinson (4), on the points of action of epinephrin and nicotin.

Following this preliminary standardization, the animals were given intravenous injections of gland extracts, and, after the blood-pressure had returned to a constant level, the reactions were again determined.

Various gland preparations were employed—fresh saline and glycerine extracts of the pancreas of the animal on the table, similar extracts of the pancreas of other dogs, commercial pancreatic extracts, principally the *holadin* of Fairchild, and saline extracts of salivary glands. Having convinced ourselves that the commercial material gave results comparable in a qualitative way with those of the other pancreas extracts, holadin was used in most of the subsequent experiments. Quantitatively

however, it proved distinctly more potent than equivalent doses of fresh saline extracts of the gland.

The tendency of the extracts of most glandular tissues, when introduced intravenously, to produce a fall in the arterial pressure has been amply demonstrated. Whether this behavior is to be attributed to the cholin content of certain of these extracts; or, in the case of the pancreas, to the action of a hormone antagonistic to epinephrin; or is to be regarded as a manifestation of protein sensitization; or finally, is due to other, still unrecognized, factors, does not concern us in this place. Suffice it to say that the characteristic depressor effect of these substances was obtained with all of the pancreas, and with some but not all of the salivary gland, preparations; and that only the data derived from those which gave such a hypotensive result are included in this report.

In addition to the fall in arterial pressure following the injections there was observed a primary, slight pressor, effect; a gradual return to the normal tension following the depression, except in the case of extremely large doses, which caused a permanent hypotension; a fairly constant direct relationship between the dose introduced and the pressure fall; an acceleration of the heart beat; and an increase in the respiratory rate which persists even after the pressure and pulse have returned to the normal.

The commercial pancreas extracts employed in this study were prepared by macerating carefully weighed amounts of the powder in distilled water for twenty minutes, after which the mixture was doubly filtered and diluted to the desired concentration. A dose of 1 cc. of a 1 : 50 saline solution of the extracts was found adequate to produce a well marked fall in blood-pressure (fig. I).

Small doses, it was noted, did not affect the standard reactions to epinephrin, tracings due to the injection of the latter, before and after the introduction of the extracts, being practically alike. With heavy pancreas doses, i.e., 1 cc. of a 1: 50 solution, on the contrary, epinephrin failed to produce as complete a response as before.

In figure II are presented comparative tracings showing the reactions to epinephrin and nicotin before and after holadin injections. Graphs 1 and 2 are the reactions to 1.0 cc. epinephrin. An average normal blood-pressure of 120 mm. was maintained during the five determinations. Before the introduction of the pancreas extract (graph 3), epinephrin caused a pressor effect amounting to 28 mm. (graph 1). The reaction to the same dose after the pancreas injection is seen in

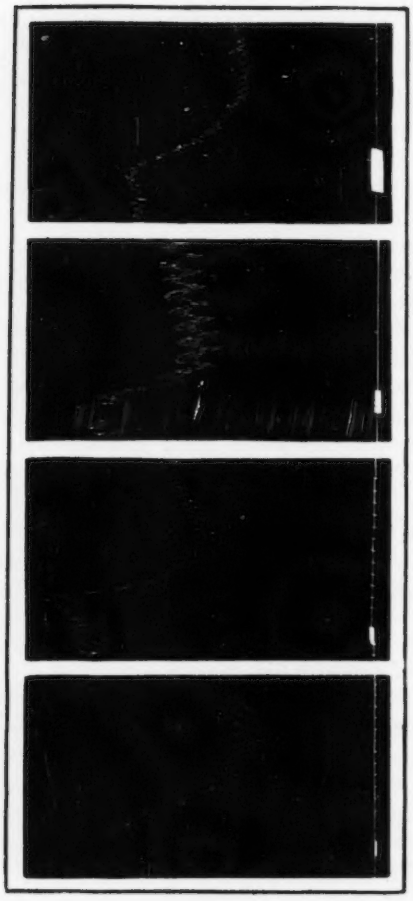

Fig. I. Tracings showing the effect upon blood-pressure of various pancreas extracts introduced intravenously. (1) Dog 8—Reaction to 1.0 cc. holadin, 1 : 50. (2) Dog 5—Effect produced by 1.0 cc. pankreon, 1 : 50. (3) Dog 9—Reaction to 2.0 cc. pankreatin, 1 : 25. (4) Dog 10—Curve following introduction of 1.0 cc. glycerine extract of dogs' pancreas. Blood pressure from femoral artery. Time intervals represent two seconds.

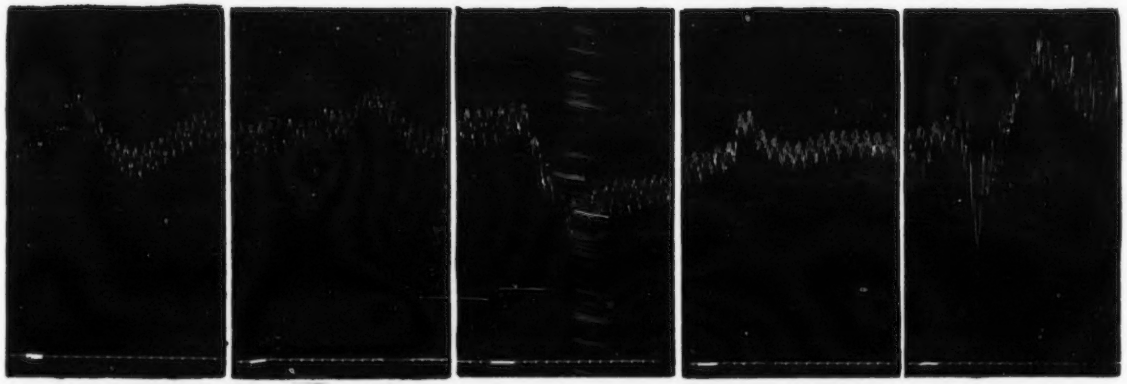

Fig. II. Tracings showing the effect of pancreas extracts upon the epinephrin and nicotin reaction. (1) Dog 3—Reaction to 1.0 cc. adrenalin, 1 : 50,000. (4) Tracing obtained from injection of 1.0 cc. nicotin, 1 : 2000. (1) and (4) are before introduction of 1.0 cc. holadin, 1 : 50. (3) Response to 1.0 cc. holadin 1 : 50. (2) Reaction to 1.0 cc. adrenalin. (5) Reaction to 1.0 cc. nicotin. (2) and (5) are after the holadin injection. Blood pressure taken from femoral artery. Time, 2 seconds.

graph 2; the rise is 22 mm.—a loss of 6 mm., or more than 20 per cent as compared with graph 1.

Similar comparative tracings showing the behavior of nicotin are seen in graphs 4 and 5. In the former there is a pressor effect of 16 mm. and in the latter of 48 mm. The increased response to nicotin, in this case amounting to 300 per cent., is characteristic, though so pronounced a sensitization is not always observed. The pancreas injection caused a depression of 50 mm., the curve returning to the normal at the end of six minutes.

In view of the well-known tendency of many other gland extracts to cause hypotension upon intravenous injection, Ringer solution extracts of the submaxillary glands were employed in a small series of animals. The results were not constant either in respect to their hypotensive action, or as regards their augmenting the response to nicotin. Those extracts, however, which were most active in depressing the arterial pressure generally produced the most marked augmentation of the nicotin reaction.

We may summarize our work as follows: Extracts of the pancreas (glycerine, saline and commercial, and particularly the latter) when introduced intravenously into dogs, cause as a rule a pronounced fall in blood-pressure, associated with an acceleration of the pulse and respirations. Repeated injections of these extracts, in a dosage sufficiently large, produce a gradually diminishing response to standard epinephrin injections. A single dose of the preparations e.g., 1 cc. of a 1: 50 solution, brings about a marked augmentation in the reaction to nicotin, which in many cases amounts to 300 per cent or more. Saline extracts of the submaxillary gland exhibit a similar, though not so constant, behavior.

As stated earlier in the paper considerable difference was noted in the depressor potency of various preparations used. A similar difference was observed in the augmentation of the nicotin reaction. This correlation suggests that the essential feature in the experiments is the hypotension. Possibly, the augmentation of sympathetic irritability is due to the cause postulated in another paper from this laboratory, i.e., a partial anemia of the medullary centers (5). The effect may be due, on the other hand, to action upon the sympathetic cells directly. The matter remains to be determined by subsequent research.

The reduction in the epinephrin reactions when larger doses were used indicates that a partial paralysis of the peripheral vascular musculature occurred. This conclusion follows, moreover, from the fact that low blood-pressure coincided with augmented vasomotor irritability.

The peripheral vascular depression suggests that due to foreign protein poisoning or to anaphylactic shock. Dose for dose, however, our pancreas preparations were distinctly more potent than peptone solutions. The obvious difference from the anaphylactic reaction is that there was no reason to suppose that previous sensitization had occurred. Just what element, if any, is common to the three cases is an open question.

SUMMARY

Various pancreas and salivary gland preparations caused a vascular depression. This was associated with a decreased reaction to epinephrin, but with an augmented reaction to nicotin. Such extracts cause therefore, an augmented irritability of the vasoconstrictor centers.

We take pleasure in acknowledging our debt to Prof. R. G. Hoskins for his assistance in this research.

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THE ORIGIN OF THE ANTIBODIES OF THE LYMPH

FRANK C. BECHT AND ARNO B. LUCKHARDT
From the Hull Physiological Laboratory of the University of Chicago

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The origin of the antibodies of the body fluids—lysins, agglutinins, and opsonins have been the subject of very extended study. Most of these studies have been confined to blood only, but of late years the other fluids have come in for a share of the observation (1), (2), (3). In work published in earlier papers (2), (3), it was shown that if the six body fluids of experimental animals most readily accessible, were arranged in order of decreasing concentration, the following series would be formed: serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humor, cerebrospinal fluid; but occasionally the order had to be reversed in the case of the last two. Clinical observations made with the Wassermann reaction show that in some cases the antibody against syphilis may be more concentrated in the cerebrospinal fluid than in the serum, at least, the cerebrospinal fluid shows a binding of complement when the serum does not. Such cases were never observed in our work on experimental animals.

With the observation of this apparently general rule of concentration of antibodies, it was apparent that two explanations of the presence of antibodies in the fluids of the body are possible; the antibodies may be formed in the blood, or reach the blood from extra vascular sources, and then pass into the lymphs and other body fluids; or, they may be formed in the lymph, or in some extra vascular cells and poured into the lymph and, then make their way into the blood stream, where the concentration becomes greatest by loss of water, by secretion, lymph formation, etc. If the first be the true condition, then in the passively immune animal, we should be able to trace the passage from blood to lymph with the true relation between concentrations as laid down by the rule; if the latter is the case, in the passively immune animal the passage from blood to lymph might be difficult and would not obey the rule laid down.

With these points in mind, the passage of antibodies from the serum into the body fluids of a normal dog, rendered passively immune by

cross circulation with another dog highly immune to some antigen was undertaken. Our method of cross circulation consisted in placing a paraffined cannula in both central and peripheral ends of the cut carotid arteries of both animals. The central end of the carotid of the normal dog was then connected with the peripheral end of the carotid of the immune dog by means of a paraffined rubber tube filled with warm 0.9 per cent NaCl solution, and *vice versa*. All clamps were then removed and the peripheral end of the carotid in each case held in the fingers in order to be sure that the blood had not coagulated in the tubing. Although no anticoagulants were used, no particular difficulty with coagulation was experienced. Cross circulation was employed rather than bleeding the normal dog dry and refilling the vessels by transfusion from an immune dog, because, while the cross circulation method did not give as high a degree of passive immunity as the transfusion method would have given, it did not at any time alter the blood pressure conditions in the recipient and, therefore, it did not alter the physiological conditions under which lymph formation was taking place. Neither was plethora induced by over filling of the vessels. So far as we could determine, the passively immune dog was normal except for having yielded one-half of his own blood, to an immune dog, and having received an equal amount of immune blood in return.

The antigen used was commonly rat or goat blood or both. B. typhosus was used in several experiments. The methods used for determining the concentration of antibodies was that recommended by Hektoen (4) in an article to which the reader is referred for the details. In general all fluids were tested within thirty-six hours after withdrawal from the body, and in the interval they were kept in the ice-box. All fluids were heated at 49°C. for thirty minutes to kill complement. The hemolysins were reactivated with guinea pig complement. Fresh washed dog leucocytes from a pleural exudate induced by aleuronat were used in determining opsonins.

The results from our experiments were so concurrent that we feel that the publication of the protocol of a single experiment is sufficient, although 20 animals were used in the series.

Dog 19. Large brindle and white cur; weight 19 k.

May 21, 1910. Intravenous injection of 20 cc. 10 per cent washed rat corpuscle.
May 23, 1910. Intravenous injection of 5 cc. goat blood.
May 30, 1910. Anaesthetized with ether, trachael cannula inserted.
10.00 a.m. Samples of serum. Neck lymph and thoracic lymph collected.
10.09–10.19. Cross circulated from carotic with Dog 20.
10.20. Serum collected. Dog killed. See table 1.

TABLE 1

Dog 19. This table shows the highest dilution at which the body fluids are just able to produce the reaction of the antibody under consideration in the body fluids of the immunized animal, and in the serum just after cross circulation with the normal. The number in each column represents the highest dilution at which the reaction in question occurred. Thus 768 = lysis in dilution of 1–768.

ANTIBODY	CORPUSCLE	10.00 A.M. (NORMAL)			10.30 (AFTER CROSS CIRCULATION)
		Serum	Neck lymph	Thoracic lymph	Serum
Hemagglutinins	Rat	768	96	192	96
	Goat	768	96	192	192
Hemolysins	Goat	96304	384	1536	6144
Hemopsonins	Rat	3072	768	1536	1536
	Goat	192	48	192	96

Dog 20. Large brown cur; weight 20 k.

10.00 a.m. Animal anaesthetized with ether. Tracheal cannula.
10.00. Samples of serum. Neck lymph and thoracic lymph collected.
10.09–10.19. Cross circulated from carotid with Dog 19.
11.20, 1.20, 3.20, 5.20, 7.20 } Sample of serum. Neck lymph and thoracic lymph taken.
7.25. Animal killed with ether. Lymphs were defibrinated and placed on ice until next day. See Table 2.

TABLE 2

Dog 20. This table shows the highest dilution at which the body fluids are just able to produce the reaction of the antibody under consideration in the body fluids of the normal dog, and in the body fluids of the same animal at various intervals after cross circulation. The number in each column represents the highest dilution in which the reaction in question took place. Thus 768 = lysis in a dilution of 1 in 768.

ANTIBODY	CORPUSCLE	SERUM							NECK LYMPH							THORACIC LYMPH						
Time		10.00	10.20	11.20	1.20	3.20	5.20	7.20	10.00	10.20	11.20	1.20	3.20	5.20	7.20	10.00	10.20	11.20	1.20	3.20	3.20	7.20
Hemagglutinins	Rat	24	768	768	768	768	768	768	12		96	96	96	96	96	12		192	192	192	192	192
	Goat	0	192	192	192	192	192	192	0		0	12	24	24	48	0		0	48	48	48	48
Hemolysins	Goat	192	3072	6144	12288	12288	12288	24576	96		96	384	768	1536	1536	96		768	3072	3072	3072	3072
Hemopsonins	Rat	12	6144	6144	6144	6144	6144	6144	12		48	192	384	1536	1536	12		1536	3072	3072	3072	3072
	Goat	6	192	92	192	192	192	192			0	6	24	24	24	0		24	96	96	96	96

These results are graphically expressed in Charts 1, 2, and 3. The results show that if an animal is rendered passively immune by the introduction of immune blood under as nearly normal conditions physiologically as possible the concentration of antibodies of the lymph rises from the first. The rise is more rapid in the thoracic lymph than in the neck lymph, and the point ultimately reached is always higher in the former than in the latter, thus obeying the rule for actively immune animals. Hence, we believe that in measuring the antibody concentration in the lymphs of the passively immune, we are measuring what takes place in the actively immune animals.[1] The antibodies

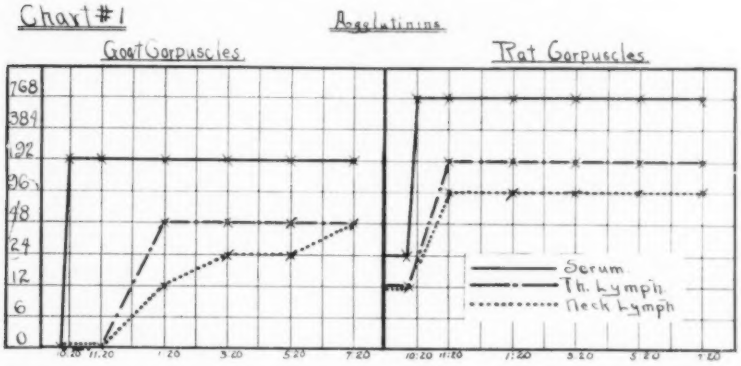

Chart 1. This chart shows the curve of the hemagglutinins in the body fluids of the normal dog after cross circulation with the immune.

reach the blood, and from that point make their way into the other body fluids by passage through the normal membranes until a certain equilibrium is reached.

CONCLUSIONS

1. The concentration of antibodies is greater in the serum than in the thoracic lymph, and greater in the thoracic lymph than in the neck lymph, not only in the actively immune animal but also in the passively immune animal; not only after equilibrium is established but at the time when active exchange is occurring.

[1] The method does not eliminate the possibility that some antibodies are added to the lymph from the tissues of the actively immune animal, nor do the authors see any method by which this phase may be studied.

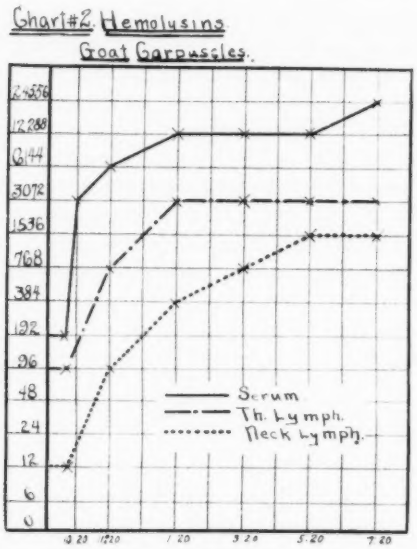

Chart 2. This chart shows the curve for the hemolysins in the body fluids of the normal dog after cross circulation with the immune.

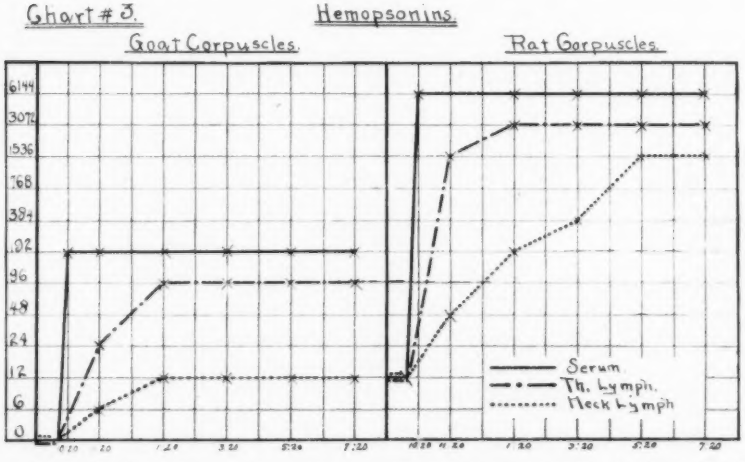

Chart 3. This chart shows the curve of the hemopsonins in the body fluids of the normal dog after cross circulation with the immune.

2. The source of the antibodies of the lymph is the blood by direct exchange from that fluid. There is no evidence that antibodies originate from the tissues and are emptied into the lymph stream at the seat of formation.

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